GENETICS

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STUDIES OF THE STREPTOMYCIN-RESISTANCE SYSTEM OF MUTATIONS IN E. COLI * †

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UNIQUE opportunities for the study of mutations, both spontaneous and induced, have been offered by what we may call the "phage-resistance system" of bacterial changes from sensitivity to resistance to bacteriophages. In studying this system it is easy to work with large numbers of bacteria (1×10^8 per Petri dish); and if sufficient quantities of phage are added, all sensitive bacteria will be eliminated whereas the resistant will survive and form colonies. A spontaneous mutation rate of about 1×10^{-8} can easily be measured by this method, and induction of mutations at a rate as low as 1×10^{-7} can be detected. It has been used in a considerable amount of research dealing with spontaneous mutability and mutability induced by X-rays, ultraviolet rays, and chemicals. In fact, for several years it was the only known method of obtaining quantitative measurements of low mutation rates.

For some time I have been on the lookout for another system, capable of being studied by similar quantitative methods, that would serve to check and confirm results obtained with the phage-resistance system. We now have found that mutations resulting in resistance to and dependence on streptomycin, and back-mutations to nondependence, have certain advantages over phage-resistance mutations for use in quantitative studies of mutability. I will describe here the results of several studies of spontaneous and induced mutability based on this streptomycin-resistance system. Summaries of these studies have been published in the Carnegie Institution of Washington Year Books Nos. 48 and 49 (DEMEREC, WALLACE, WITKIN and BERTANI 1949; DEMEREC *et al.* 1950).

MATERIAL AND NOMENCLATURE

The experiments were made with Strain *B/r* of *Escherichia coli*, which is a radiation-resistant mutant of strain *B* (WITKIN 1947). Its resistance to radiation was a distinct advantage in the irradiation experiments, for it permitted treatment with higher doses. In one experiment we used strain *B/r/6*, a mutant of *B/r* that is resistant to phage 6 of the T series. Several other phages of the T series were used in tests made to detect and eliminate possible contaminations.

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Dihydrostreptomycin sulfate was used in the preparation of media containing the desired concentrations of streptomycin.

This paper will employ the system of nomenclature described previously (DEMEREC and FANO 1945). The symbol *B* represents the original strain of *E. coli*, a bar (/) indicates " resistant to," and the symbols following the bar stand for materials to which the mutant of strain *B* is resistant. Thus *B/S* is a mutant of strain *B* resistant to streptomycin; *B/Sd* is a mutant not only resistant to but dependent on streptomycin. Numbers indicate phages of the T series (*T1, T2, . . . T7*); *B/1* is thus a mutant of strain *B* resistant to phage *T1*. Since *B/r* was the strain used in all these experiments (except number 13 of table 2), the mutants studied were actually *B/r/S* and *B/r/Sd*. For the sake of simplicity, however, the " *r* " symbol will be omitted throughout the paper. Capital *R* is the symbol for reversions (back-mutations) from dependence (*B/Sd*) to nondependence.

ACTION OF STREPTOMYCIN ON E. COLI

It was of interest first to determine the toxic effect of streptomycin on *E. coli*. Previous experiments (DEMEREC 1948) had shown that concentrations up to 1.5 micrograms per milliliter do not kill bacteria grown in an aerated broth culture. With increasing concentrations between 1.5 and 20 μg, the number of survivors decreases rapidly to about 6 per 10^8. Beyond this point, continued increases have very little further effect on the proportion of survivors. The earlier experiments had shown that bacteria surviving concentrations higher than about 4 μg per ml are mutants that have greater resistance to streptomycin than the parent strain. Survivors of concentrations of 20 μg are regarded here as completely resistant.

That streptomycin kills bacteria before a division is accomplished is suggested by the experiments summarized in table 1. In these experiments sam-

TABLE 1

Percent of survivors among resting bacteria kept for various periods in broth containing different concentrations of streptomycin at 37° C.

Concentration of streptomycin (μg/ml)	% of survivors after exposures of						
	60	80	100	120	140	180	24 hours
	minutes						
3	93	100	79	67			< 0.6*
4	100	78	84	67			< 0.7
5	100	81	85	78			< 0.8
6	93	85	65	37			< 0.6
7		94	84	59	47	33	< 0.3
10		76	65	62	41	20	< 0.3
12		76	68	51	30	11	< 0.4
15		88	69	53	32	25	< 0.4
20		66	46	38	26	15	< 0.3

* Living bacteria were not found in samples originally containing between 130 and 368 bacteria.

ples of about 16,000 bacteria were placed in tubes containing 5 ml of broth to which streptomycin had been added in the quantities indicated. The tubes were incubated at 37°C, and at stated intervals 0.1-ml samples were plated to determine the number of living bacteria present in the cultures. Results showed that the number of living bacteria decreased with the length of exposure to streptomycin. Microscopic observations of bacteria plated on broth agar containing 5 μg of streptomycin per ml did not reveal any divisions in the bacteria so exposed. From this it can be inferred that killing by streptomycin does not require division of the bacteria, and that in this respect streptomycin differs from penicillin.

SPONTANEOUS MUTABILITY

Rate of mutation to streptomycin resistance. LURIA and DELBRÜCK (1943) developed two methods for measuring low mutation rates in bacteria. Both methods use a large number of small independent cultures, started from inocula small enough not to include any mutants, and register the mutants present in these cultures after bacterial growth has stopped. One method calculates mutation rate from the percentage of cultures containing no resistant mutants, the other from the average number of mutants per culture. A prerequisite of the second method is that the mutant bacteria divide at the same rate as nonmutants. Since this is not true of streptomycin-resistant mutants, only the first method can be used in our work. The formula yielding the mutation rate (a) per bacterial generation is:

$$a = -\frac{1.6}{N} \log_{10} p$$

in which N represents the average number of bacteria per culture, and p the percentage of cultures containing no resistant mutants.

The data of four experiments are given in table 2. Two of these used strain *B/r*, one strain *B/6*, and one strain *B/r/1*. To isolate resistant mutants, the whole content of each of the independent cultures was plated into agar containing 25 μg of streptomycin per ml. The results of these experiments indicate that the mutation rate is about 1×10^{-9} per bacterial generation, and that it is not appreciably affected by the strain used in tests.

Properties of resistant mutants. Resistant mutants that originated independently were found to include several distinct types. The most striking differentiation among them was with regard to dependence on streptomycin. An analysis of 208 showed that 124, or 60 percent, were dependent on streptomycin for their growth; that is, they were able to grow fully only on medium containing streptomycin, and passed through only a few divisions on medium lacking streptomycin. Other conditions being equal (*i.e.*, number of bacteria in culture, concentration of streptomycin in the parent culture), the number of divisions undergone by dependent bacteria on medium lacking streptomycin is a constant property of individual mutants, which vary greatly in this respect.

TABLE 2

Distribution of numbers of streptomycin-resistant mutants in a series of similar cultures in 1 ml synthetic medium.

Experiment No. and Strain	11 B/r	12 B/r	13 B/6	14 B/r/1
Resistant bacteria	No. of cultures			
0	25	56	19	63
1	17	22	25	18
2	12	10	21	6
3	8	6	21	0
4	2	5	9	2
5	2	0	2	1
6–10	3	4	4	0
10–50	1	0	1	3
50–100	0	0	0	1
> 100	1	0	0	9
Total	71	103	102	103
Aver. no. bacteria ($\times 10^9$)	1.35	0.48	0.44	0.28
Mutation rate ($\times 10^{-9}$)	0.73	0.91	2.6	1.3

Different dependent (*B/Sd*) and nondependent resistant (*B/S*) mutants differ considerably in their rates of growth. Many of them are "slow growers"—that is, types that when plated on broth-agar medium require 48 hours or longer to develop colonies of the size reached by the original strain in 24 hours.

Growth rates of nine *B/Sd* and two *B/S* strains were studied by inoculating broth with bacteria, incubating the cultures at 37°C, and removing samples at intervals to assay the number of bacteria. The concentration of streptomycin in the medium in which the *B/Sd* strains were grown was 10 μg per ml. The lag period—that is, the time required for doubling the original number of bacteria—varied in these nine strains from 110 to 210 minutes; and the time taken for completion of one division varied from 45 to 100 minutes. Since the lag period for the *B/r* strain is 60 minutes, and a division is completed in about 20 minutes, it is evident that these *B/Sd* mutants, selected at random, required a considerably longer time both to start growing and to complete a division. The two *B/S* strains were grown in broth without streptomycin and in broth containing 10 μg of streptomycin per ml. The presence or absence of streptomycin made no difference in the growth of these two strains. For both, the lag period was about 65 minutes and the division time about 25 minutes.

Three other mutant strains of *B/S* were grown in mixtures with *B/r*, to test their survival values when competing with the original strain. Results of these tests, given in table 3, show that all three were at a disadvantage as compared with *B/r*.

Observations made on these five *B/S* and many *B/Sd* mutants indicated that none of them grew as well as strain *B/r* in medium without streptomycin.

TABLE 3

Results of experiments in which B/r and B/S bacteria were grown together in broth cultures.

B/S Mutant No.	Beginning of experiment		End of experiment	
	No. of bacteria	Proportion B/r : B/S	No. of bacteria	Proportion B/r : B/S
5	6.6×10^3	5.5 : 1	3×10^8	130 : 1
	1.13×10^4	48 : 1	3.9×10^8	1.6×10^3 : 1
6	7.1×10^3	3.4 : 1	2.5×10^8	2.5×10^3 : 1
	1.13×10^4	83 : 1	2.3×10^8	1.6×10^4 : 1
7	2.6×10^4	1 : 1.3	2.3×10^8	10 : 1
	2.3×10^4	77 : 1	2.3×10^8	2×10^3 : 1

In such a medium the *B/Sd* bacteria divided only a few times and the *B/S* grew at a slower rate.

Back-mutations from dependence to nondependence. When bacteria of a *B/Sd* strain are plated on agar containing no streptomycin, they undergo one or several normal divisions, and then in some strains pass through several additional abnormal divisions, giving rise to elongated filaments or " snakes," which increase in length up to about ten times the length of a bacterium and then stop growing. Observations made by BERTANI (1951) indicate that mutations to nondependence occur during the normal divisions but not in the filaments. Mutant cells are able to grow normally on the broth-agar medium, and form colonies.

The number of divisions is influenced by several environmental factors, chiefly by the amount of streptomycin present in the medium in which the bacteria were cultured, and to a lesser degree by the number of bacteria plated on the agar. If these conditions are kept constant, the average number of divisions for any one strain will also be constant. The different strains, however, show striking differences with regard to the number of normal and abnormal divisions they pass through.

The dependent strain *B/Sd-4* was used in our studies of spontaneous and induced back-mutations (reversions). Various biological properties of this strain have been investigated in detail by BERTANI (1951). Here I will give a brief summary of the procedure followed in experiments to be mentioned in this paper.

B/Sd-4 bacteria were grown under aeration, either in broth or in a synthetic medium (M9) containing 10 μg of streptomycin per ml. After the bacteria were washed—by centrifuging, decanting the supernatant, shaking up the pellet, and repeating the same processes again—they were plated on broth-agar medium containing no streptomycin. In experiments to observe back-mutations, care was taken to plate no more than 5×10^7 living bacteria per Petri dish. Back-mutants were scored after 72 hours of incubation. In doubtful cases, the observations were checked with a low-power wide-field binocular microscope. In preliminary tests, scoring had been done after 48, 72 and

96 hours of incubation. An increase in number of back-mutants was observed between the 48-hour and 72-hour scorings, but there was no further increase at 96 hours.

Under the circumstances described above, *B/Sd-4* bacteria pass through 2.7 normal divisions before beginning the formation of snake-like filaments. After 72 hours of incubation, a plate on which about 5×10^7 bacteria have been spread will show, in addition to a very thin layer of growth on the surface, a few scattered colonies representing mutants. A count of these colonies will determine how many mutations to nondependence have occurred; and if the number of plated bacteria is known the mutation rate per specified number of bacteria can be calculated. An estimate of the mutation rate per bacterial division can be obtained by dividing the mutation rate per plated bacteria by a factor of 6, which represents an estimate of the increase in number of *B/Sd-4* bacteria raised on 10 μg of streptomycin per ml when plated on medium without streptomycin. The factor is greater than 6 if the bacteria are raised in a medium containing more than 10 μg of streptomycin per ml.

The rate of spontaneous mutation of *B/Sd-4* to nondependence is about 1.4×10^{-8} per bacterial generation. Detailed data are given by BERTANI (1951).

MUTATIONS IN *B/Sd-4* INDUCED BY RADIATIONS

Ultraviolet rays. Bacteria raised in an aerated culture containing broth and 10 μg of streptomycin per ml were used in these experiments. The procedure was as follows:

(1) Bacteria were washed in saline to remove excess streptomycin.
(2) Bacterial suspension was assayed by plating, to determine concentration.
(3) Five control plates were started by plating 0.1 ml of a 10^{-3} dilution of bacterial suspension on broth-agar plates, to determine spontaneous mutation rate.
(4) A thin layer (about 7 ml) of bacterial suspension in saline was placed in the bottom part of a Petri dish and exposed to ultraviolet rays from a germicidal lamp (2537 Å), with continuous shaking during exposure.
(5) A sample of the irradiated suspension was assayed to determine the proportion of bacteria surviving treatment. Dosage was calculated from the survival ratio (using the chart published by DEMEREC and LATARJET 1946) and the exposure time.
(6) 0.1-ml samples of the irradiated suspension were plated on broth-agar plates to determine the frequency of mutations induced in the treated bacteria.
(7) Assay plates were scored after 24 hours of incubation at 37°C; and control and experimental plates were scored after 72 hours of incubation.
(8) Frequency of induced mutations per 10^8 bacteria plated was computed.

Microscopical examination of irradiated *B/Sd-4* bacteria revealed that they divide on broth-agar medium to the same extent as nonirradiated bacteria, so that the same factor can be used for computing mutation rate per bacterial division.

Data on the numbers of mutations induced by different doses are given in table 4 and presented graphically in figure 1. These results will be considered in the Discussion.

X-rays. Except for the actual treatment of bacteria, the procedure used in X-ray experiments was identical to that used with ultraviolet. Treatment was given in small glass test tubes, each containing 4.5 ml of bacterial suspension. As a rule, nine test tubes were exposed simultaneously and then removed one

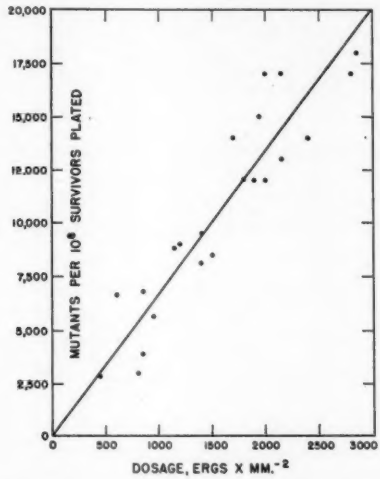

FIGURE 1.—Plot of data on mutants (both sensitive and resistant) induced by treating strain *B/Sd-4* of *E. coli* with different doses of ultraviolet rays (2537 Å).

by one at intervals representing the desired length of exposure. Intensity of the X-rays was measured with a Victoreen dosimeter, and the dosage was determined by length of exposure. The X-ray machine was run at 140 kv and 8 ma. At the exposure distance used, the dose was about 30 roentgens per second.

The results of the X-ray experiments are summarized in table 5 and presented graphically in figure 2.

MUTATIONS IN *B/r* INDUCED BY ULTRAVIOLET RADIATION

Extensive testing showed that induced mutants cannot be observed if radiation-treated bacteria are plated on or in medium containing streptomycin immediately after treatment. This fact indicated that irradiated bacteria have to undergo division before the mutations induced by the radiation can be ex-

TABLE 4

Summary of experiments with B/Sd-4 on relation between dose of ultraviolet radiation (2537 Å) and number of induced nondependent mutants (both streptomycin-sensitive and streptomycin-resistant).

Exper. No.	Survivors (%)	Dosage (ergs)	No. of bacteria		No. of mutants	
			Per plate	Total	Total	Per 10^8 plated bacteria
6-3	53.4	450	4.8×10^6	1.9×10^7	565	2,850
8-2	41.5	600	5.8×10^5	1.7×10^6	114	6,700
5-3	29.4	800	2.1×10^6	6.3×10^6	192	3,000
6-4	22.2	850	2.0×10^6	6.0×10^6	408	6,800
3-3	22	850	2.5×10^7	1.25×10^9	4886	3,900
4-3	18.2	950	2.8×10^6	8.4×10^6	476	5,700
6-5	9.7	1150	8.7×10^5	2.6×10^6	229	8,800
8-3	7.9	1200	5.5×10^5	1.1×10^6	100	9,100
4-4	4.2	1400	3.3×10^6	6.6×10^6	629	9,500
5-4	4.1	1400	1.5×10^6	3.0×10^6	246	8,200
7-3	2.8	1500	2.6×10^6	1.3×10^7	1099	8,500
6-6	1.3	1700	1.2×10^5	6.0×10^5	85	14,000
7-4	0.76	1800	7.1×10^5	3.6×10^6	441	12,000
8-4	0.56	1900	7.8×10^5	2.3×10^6	276	12,000
3-4	0.45	1950	1.0×10^6	2.0×10^7	297	15,000
4-5	0.32	2000	5.0×10^5	1.0×10^6	172	17,000
5-5	0.35	2000	2.5×10^5	5.0×10^5	62	12,000
6-7	0.19	2150	1.7×10^4	8.5×10^4	11	13,000
7-5	0.15	2150	1.4×10^5	5.6×10^5	97	17,000
8-5	0.05	2400	7.0×10^4	2.1×10^5	30	14,000
7-6	0.012	2750	1.1×10^4	5.5×10^4	15	27,000
5-6	0.011	2800	8.0×10^3	4.8×10^4	8	17,000
4-6	0.009	2850	1.4×10^4	2.8×10^4	5	18,000

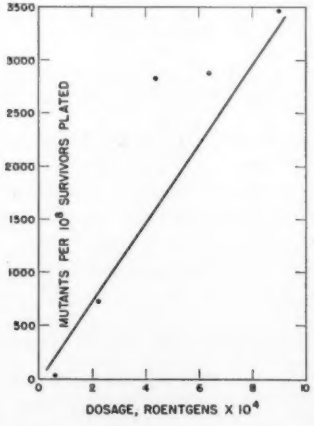

FIGURE 2.—Plot of data on mutants (both sensitive and resistant) induced by treating strain *B/Sd-4* of *E. coli* with different doses of X-rays.

TABLE 5

Summary of experiments with B/Sd-4 on relation between X-ray dosage and number of induced nondependent mutants (both streptomycin-sensitive and streptomycin-resistant).

Exper. No.	Dosage (roentgens)	Survivors (%)	No. of bacteria		No. of mutants	
			Per plate	Total	Total	Per 10^8 plated bacteria
3-3	6,000	52.5	1.6×10^7	3.2×10^7	1464	46
1-3	22,000	8.85	2.0×10^6	2.0×10^7	147	735
3-5	44,000	0.66	2.0×10^7	4.0×10^7	1128	2820
1-4	46,000	0.491	1.1×10^6	1.1×10^7	129	1170
3-6	64,000	0.063	1.9×10^6	1.6×10^7	446	2890
2-6	68,000	0.04	1.5×10^6	1.5×10^7	311	2080
2-7	90,000	0.00306	1.14×10^5	1.1×10^6	38	3460

pressed. The following technique was developed for studies of such induced mutations:

(1) The culture was assayed by plating, to determine the concentration of bacteria. When ultraviolet treatment was to be given, bacteria were cultured in transparent synthetic medium (M9).

(2) A thin layer of bacterial suspension was irradiated, with continuous shaking.

(3) A sample of the irradiated suspension was assayed by plating to determine the proportion of survivors.

(4) Samples were plated on broth agar containing 25 μg of streptomycin per ml, to determine the " background " number of resistant mutants present.

(5) Samples were plated on plain broth agar and incubated for a certain period (2 hours or longer) so that the bacteria would divide. Since the lag period varies with dosage, the incubation period was adjusted accordingly.

(6) After incubation, the plates were refrigerated to stop growth.

(7a) Growth of the bacteria during incubation was determined on 3 plates by washing with 10 ml of broth and assaying the suspension.

(7b) Onto each of the remaining plates was poured 4 ml of soft agar (0.7 percent) containing 200 μg of streptomycin per ml. After the agar had set these plates were kept at 16°C for 2 hours, allowing time for the streptomycin to act on sensitive bacteria. The plates were then incubated at 37°C, and after 48 hours the colonies were counted. Under these circumstances, only resistant bacteria survive and form colonies; therefore, after the number of background mutants had been deducted, the counts showed the number of induced mutants that were present after the treated bacteria had multiplied a definite number of times.

A summary of the data of these experiments with ultraviolet treatment is given in table 6. Here, again, dosage was calculated from the percentage of

survivors. A fresh culture of bacteria was used for each experiment. Examination of the table shows that the total number of mutants in any one experiment depended not only on the dose used in treatment but also on the subsequent stage of division at which mutants were scored. Since it would be a very difficult and laborious process to obtain material that had received different treatments but undergone the same number of divisions after treatment, the material and method described here are not suitable for quantitative studies of induced mutations. In this case it is not feasible to work with "endpoint" mutants, as has been done successfully in studies with phage resistance

TABLE 6

Summary of experiments with B/r on induction of mutations to streptomycin resistance by ultraviolet rays.

Exper. No.	Survivors (%)	Dosage (ergs)	Incubation (hours)	No. of bacteria			No. mutants per 10^8		
				Plated	Washed	Increase (×)	Total	Plated	Washed
14-3	22.4	800	2	1.2×10^8	1.46×10^8	1.07	50	39.7	34.3
-4			4	1.02×10^8	1.24×10^8	1.2	346	339	279
-5			5	1.02×10^8	6.04×10^8	5.9	702	687	116
15-3	12.9	1000	2½	6.0×10^7		0	104	174	174
-4			3½	6.0×10^7	1.44×10^8	2.4	336	560	233
12-2	13.5	1000	3	2.24×10^7	3.14×10^7	1.4	116	535	383
7	14.5	1000	3½	5.8×10^7	1.77×10^8	3	341	588	192
16-2	11.5	1050	2½	9.36×10^7		0	167	179	179
-3			3½	6.02×10^7	9.12×10^7	1.3	347	576	380
-4			4½	1.87×10^7	2.0×10^8	10.7	182	1000	91
-5			5½	6.28×10^6	9.0×10^8	128	130	2167	14.4
3	10.6	1100	3	1.7×10^7	3.6×10^7	2.1	632	3750	1770
13-4	11	1100	2	6.18×10^7		0	83	134	134
-5			3	5.15×10^7	6.4×10^7	1.28	304	590	475
-6			4	5.15×10^7	6.5×10^8	13	1221	2368	1882
4	8	1200	3	2.05×10^7	2.4×10^7	1.2	165	825	688
5	5.6	1350	3	2.3×10^7		0	149	650	1120
20-3	2	1600	4	7.08×10^7	1.0×10^8	1.42	410	580	410
-4			4½	5.9×10^7	3.6×10^8	6.08	623	1056	174
12-3	1.34	1700	3½	6.66×10^6	7.2×10^6	1.1	40	610	555
19	1.35	1700	4	4.5×10^7	4.8×10^7	1.07	180	400	375
			4½	4.5×10^7	1.48×10^8	3.3	361	804	244
17	0.55	1900	4	1.98×10^6	3.0×10^6	1.5	12	606	400
11	0.2	2100	3½	8.96×10^5	3.5×10^6	3.9	10	1115	286

(DEMEREC and LATARJET 1946), because of the technical difficulties of applying streptomycin to colonies of cells after they have reached a certain size. One of the main requirements of the method is that cells belonging to different colonies not be mixed together when the streptomycin is applied; and a suitable way of preventing this, after the number of cells per colony has increased beyond a certain stage, has not yet been found.

Among the many experiments done by us, there were a few that showed a similar increase in number of bacteria between the treatment and the application of streptomycin; in these, the factor of increase varied between 1.2 and

1.5. The data of these experiments are plotted in figure 3, which indicates a straight-line relation between ultraviolet dose and induced mutations.

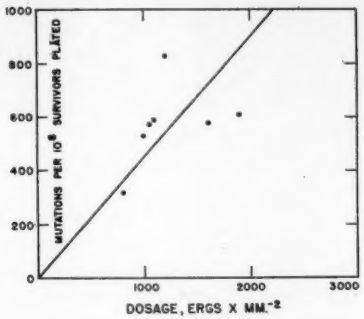

FIGURE 3.—Plot of data on mutants (both streptomycin-resistant and streptomycin-dependent) showing up after strain *B/r* of *E. coli* was irradiated with different doses of ultraviolet rays (2537 Å) and the bacteria allowed to multiply 1.2 to 1.5 times.

DISCUSSION

Studies of streptomycin-resistant mutants have revealed an interesting situation; namely, that none of the mutants investigated can compete with the original strain when grown without streptomycin. Since about 60 percent of these mutants are dependent on streptomycin, they can pass through only a few divisions without it; but even those resistant mutants that are not streptomycin-dependent seem to be at a disadvantage as compared with nonmutants. This was indicated by a study of five resistant mutants, three of which were tested in mixtures with nonmutant bacteria and two of which were studied for division rate.

Experiments with ultraviolet radiation and with X-rays showed two significant results. The first was that there are no "zero-point" mutations among the induced mutations from *B* to *B/S* and *B/Sd*, and also that there are probably none among reversions from *B/Sd*. This is in contrast to the phage-resistance system, where some of the induced mutations do not need cell division for their expression (zero-point mutants) although others require at least one cell division to take place before they show up (end-point mutants). In every irradiation experiment made in the study of induction of mutations from *B* to *B/S*, bacteria were plated, immediately after treatment, on medium containing streptomycin; therefore records of a large number of such platings are available. In no case was the proportion of mutants significantly higher than in treated controls, which offers good evidence that there were no zero-point mutants.

In experiments 15–3, 16–2, 13–4, and 13–5, listed in table 6, the numbers of mutants per 10^8 were high, although no increase in numbers of plated bacteria was detected. In these cases, however, the bacteria were incubated for 2–3 hours after treatment, and undoubtedly some of them divided, although

the number may not have been sufficiently high to be detected by the method used in the experiments. Therefore these data are not in disagreement with the conclusion that zero-point mutations do not occur among induced *B*-to-*B/S* mutations.

If zero-point mutations occurred among induced back-mutations from *B/Sd*, they would be expected to produce colonies that could be detected after incubation of about 12–15 hours. Since no unexpectedly large proportions of early-appearing colonies were noticed in any of the experiments, it seems reasonable to conclude that zero-point mutants are not present among reversions.

Mutations from *B* to *B/S* induced by ultraviolet radiation did not follow the pattern of expression observed in delayed mutations from *B* to *B/1* (DEMEREC 1946); that is, their time of appearance did not stretch over a long period during which the total number of bacteria increased manyfold. In the case of induced *B/1* mutants, only a small proportion were expressed after the first few divisions of the irradiated bacteria, whereas the great majority showed up after later divisions; and the mutation rate per bacterium per division returned to the spontaneous level only after 10 to 12 divisions. Examination of the data presented in table 6 reveals a pattern of appearance of induced mutants that is not comparable to that observed in the *B/1* material. For example, in experiment 14, after 4 hours of incubation (14–4), when the number of bacteria had increased about 1.2-fold (*i.e.*, before all the irradiated bacteria had undergone one division), the frequency of induced mutants was 339 per 10^8 bacteria surviving the treatment. In the same experiment, after 5 hours of incubation (14–5), when the number of bacteria had increased 5.9-fold, the frequency of mutants was 687 per 10^8. Similarly, in experiment 16, after 3½ hours of incubation the bacteria had multiplied 1.3-fold and the frequency of mutants was 576 per 10^8; after 4½ hours, the increase was 10.7-fold and the frequency of mutants 1000 per 10^8; and, finally, after 5½ hours the increase was 128-fold and the frequency of mutants about 2167 per 10^8. In this last-mentioned experiment there was an indication that some induced mutants were expressed during the period when the bacteria increased from 10-fold to 128-fold, but their number was very small compared with the increment of *B/1* mutants during the comparable period. More extensive experiments carried on in connection with another study did not show any consistent increase in the frequency of induced mutants after the treated bacteria had undergone between 2 and 4 divisions.

Recently NEWCOMBE and HAWIRKO (1949) made a study of the rate of mutation from streptomycin sensitivity to streptomycin resistance in this same strain of *coli*. They calculated the mutation rate as 10^{-10} per bacterium per division, which is about ten times lower than the rate observed in these experiments. The only apparent difference between the methods employed is that NEWCOMBE and HAWIRKO used a medium containing 64 μg of streptomycin per ml to select mutants, whereas we used a concentration of 25 μg per ml.

The second important finding brought out in this study is the straight-line relation between ultraviolet dose and number of induced mutants. This applies

to induced mutations from *B/Sd* to *R* (back-mutations to nondependence) as well as to those from *B* to *B/S*. It is quite different from the situation found in the phage-resistance system, where a multiple-hit curve was indicated for the relation between ultraviolet dose and induced mutation rate (DEMEREC and LATARJET 1946).

SUMMARY

Experimental results indicate that the rate of mutation in strain *B* (*B/r*) of *E. coli* from sensitivity to complete resistance to streptomycin is about 1×10^{-9} per bacterium per division. About 60 percent of the resistant mutants are dependent on streptomycin for their growth.

The rate of mutation from *B* to *B/S* (resistance to streptomycin) can be studied with considerable precision, since the work can be carried out with very large numbers of bacteria. The same is true for the study of back-mutation (*R*, equals reversion) from streptomycin dependence (*B/Sd*) to nondependence. The mutation rate in the mutant strain *B/Sd-4* is about 1.4×10^{-8} per bacterium per division.

Mutations from *B* to *B/S*, as well as from *B/Sd* to *R*, may be induced by ultraviolet rays and by X-rays. The dose–mutation rate relation is represented by a straight line for both radiations.

It is necessary for at least two to four cell divisions to take place before the induced mutations are expressed; " zero-point " mutants do not appear in the streptomycin-resistance system.

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A METHOD FOR DETECTION OF MUTATIONS, USING STREPTOMYCIN DEPENDENCE IN ESCHERICHIA COLI *

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IN studies of induced mutation it is particularly desirable to use well-controlled methods for the detection of mutations. MULLER's introduction of the *CIB* method for detecting lethal mutations in Drosophila first opened this field of study and much work has been done with this organism in spite of the laborious technical procedures required. Microorganisms offered a simpler material for this kind of work. DEMEREC's studies (DEMEREC, WALLACE, WITKIN and BERTANI 1949) of spontaneous and radiation-induced mutation in the streptomycin resistance system in *Escherichia coli* indicated that a suitable test material for induced mutation might be the back-mutation from streptomycin dependence to nondependence. The present paper is concerned with the development of such a test, based on plating streptomycin-dependent bacteria on agar containing no streptomycin. On this medium, nondependent cells (back-mutants) are able to form visible colonies; whereas the dependent cells divide a few times (residual growth), thus increasing the chances for back-mutation. If all or most back-mutations occur after plating, exposure to a mutagenic agent before plating can be expected to increase the number of back-mutants. We shall examine in this paper the factors that affect the results obtained with this technique and that must be controlled in quantitative studies of induced mutation.

MATERIALS AND METHODS

Streptomycin-dependent bacterial strains were first described by MILLER and BOHNHOFF (1947). Dependent strains have since been isolated from a number of bacterial species (see WAKSMAN 1948, 1949).

In *Escherichia coli,* dependent mutants are found among the resistant mutants that can easily be obtained from sensitive strains by suitable selection techniques (NEWCOMBE and HAWIRKO 1949; DEMEREC *et al.* 1949). The strains used in our work were isolated from the sensitive strain *B* of *E. coli;* they will be referred to by the symbols *B/Sd* or simply *Sd* (= streptomycin-dependent). *Sd* strains of different mutational origin will be distinguished by means of a number; most data concern *Sd-4* (obtained from DEMEREC), although a few observations have also been made on *Sd-26, Sd-3,* and other

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dependent strains. Sensitive mutants derived by back-mutation from dependent strains will be called *R* (= reversion), to distinguish them from *B*, the original sensitive strain, to which they are phenotypically similar; resistant, nondependent mutants derived from dependent strains will be designated *RS*. In some of the experiments two strains marked with mutations for phage resistance were used: *B/Sd-4/1,3,4,5* and *B/Sd-4/3,4*. The first is resistant to phages *T1, T3, T4, T5*; the second to *T3* and *T4*.

The dependent strains were cultivated in nutrient broth containing streptomycin (usually 25 micrograms per milliliter). Colony counts (assays) were made on streptomycin agar (nutrient agar to which 50 μg of streptomycin per ml was added just before pouring). Streptomycin sulfate and dihydrostreptomycin sulfate were used; no essential difference was observed.

The plate-"washing" technique described by BEALE (1948) was used to determine the total number of bacteria present on a plate after incubation; each plate was "washed" once with 10 ml of broth. On the average, 84 percent of the bacteria present on a plate can be recovered.

A nephelometer designed by UNDERWOOD and DOERMANN (1947) was used to measure the generation time of different strains in the exponential phase.

RESIDUAL GROWTH OF *Sd-4* IN THE ABSENCE OF STREPTOMYCIN

General properties of Sd-4. The generation time of *Sd-4* in broth (aerated culture) containing 20 μg of streptomycin per ml, measured nephelometrically at 37°C, is approximately 34 minutes. The final titer reached by *Sd-4* in broth does not change with concentrations of streptomycin between 1 and 100 μg per ml, and is usually between 1.5 and 3.9×10^9 bacteria per ml. As the concentration increases beyond 100 μg per ml the final titer slowly decreases.

Residual growth in liquid cultures. Sd bacteria grown with streptomycin, washed, and transferred to fresh streptomycin-free medium, do not stop growing at once, but undergo a certain number of divisions. If the inoculum is small (of the order of 10^3 cells per ml) this residual growth is very slight. If the inoculum is of the order of 5×10^7 bacteria per ml, full growth is obtained in 12 to 24 hours. Figure 1 shows an experiment of this kind. Parallel platings on plain and on streptomycin agar at the end of the experiment showed that growth was due to dependent cells and not to resistant mutants that might have overgrown the *Sd* cells.

Residual growth is affected by the concentration of streptomycin in the culture from which the inoculum is taken: the higher this concentration, the greater the amount of residual growth (fig. 2).

Residual growth on agar. Residual growth on agar has to be studied by different methods: microscopic count of bacteria per microcolony (which gives the total number of bacteria), and plate-washing technique (which gives the number of viable [2] bacteria present on the plate). The two methods sup-

[2] The word "viable" is used throughout this paper, with reference to streptomycin-dependent bacteria, to mean: "able to start dividing again when transferred to nutrient medium containing streptomycin."

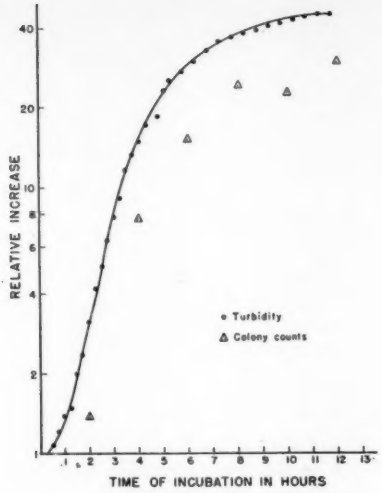

FIGURE 1.—Multiplication of streptomycin-dependent *E. coli* in broth containing no streptomycin, followed both nephelometrically and by colony counts. Inoculum: 5.9×10^7 per ml. Ordinate 1 represents the initial turbidity and the initial number of viable bacteria.

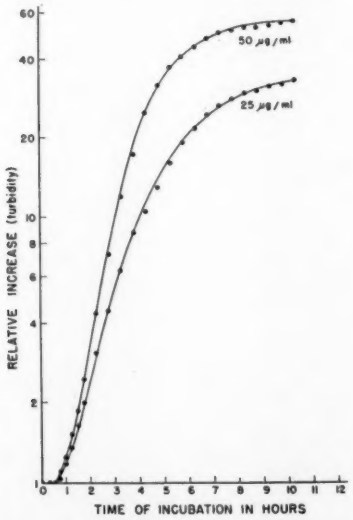

FIGURE 2.—Multiplication of streptomycin-dependent *E. coli* in broth containing no streptomycin. Heavy inocula, from cultures grown in 50 μg and 25 μg of streptomycin per ml, respectively.

ply the same kinds of information, respectively, as the nephelometric technique and the colony count in the case of liquid cultures. Figure 3 shows the extent of residual growth of dependent bacteria plated on plain nutrient agar, as observed by these two methods. A significant discrepancy is noted between the total counts and the numbers of viable cells.

Figure 4 shows the effect of crowding. For initial concentrations higher than 10^7 cells per plate, the larger the number of cells plated the greater is the extent of residual growth.

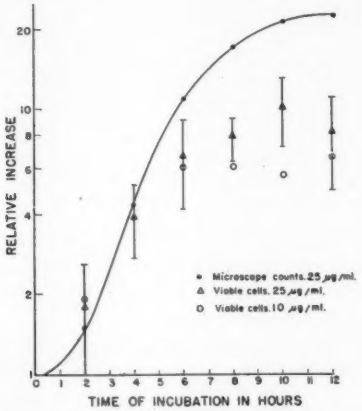

FIGURE 3.—Multiplication of streptomycin-dependent *E. coli* on agar containing no streptomycin. The curve through the solid dots represents the increase in average numbers of bacteria per microcolony, as observed microscopically; each point is based on observation of 200 microcolonies. Inoculum from a culture with 25 μg of streptomycin per ml.

The triangles give the corresponding values for viable bacteria. Inoculum as above. Data from five experiments. 1.6 to 3.8 $\times 10^7$ bacteria per plate at time 0. The limits $\pm$ 2 standard error are given. The circles give values obtained using an inoculum from a culture with 10 μg of streptomycin per ml. Data from two experiments. 1.8×10^7 bacteria per plate at time 0.

Less extensive data are available concerning the effect of previous concentration of streptomycin on residual growth on agar. Nevertheless, microscopical observations show that if cells are grown in 1 μg of streptomycin per ml and plated on plain agar, only a few are able to undergo even one cell division. Cells grown in 10 μg of streptomycin undergo fewer divisions than cells grown in 25 μg (see fig. 3).

Formation of "snakes" during residual growth. After 12 hours of incubation the number of bacteria has reached a maximum (fig. 3). Thereafter very few cell divisions occur, but cell elongation and nuclear division continue, so that the cells gradually develop into long polynucleated filaments (snakes). The cytology of these forms has been studied by DELAPORTE (1949). Snakes

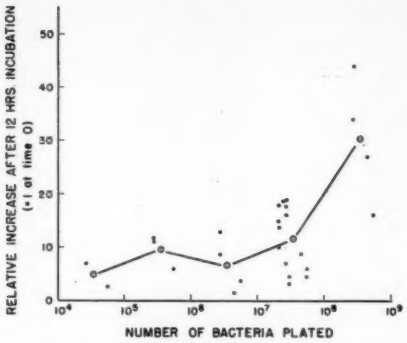

FIGURE 4.—Effect of crowding on the residual growth of streptomycin-dependent *E. coli* plated on agar. Inocula from cultures with 25 μg of streptomycin per ml. Every point represents the factor of increase in number of the cells originally plated, at the end of 12 hours' incubation, as determined by the plate-washing technique.

exhibit peculiar movements on the surface of agar, which makes it difficult to count the bacteria of a microcolony after the beginning of snake formation. It was necessary, therefore, to use a different technique for studying residual growth after more than 12 hours of incubation.

Washed dependent bacteria were plated (approximately 2×10^7 per plate) on plain nutrient agar and incubated for 12 hours at 37°C; then the bacteria

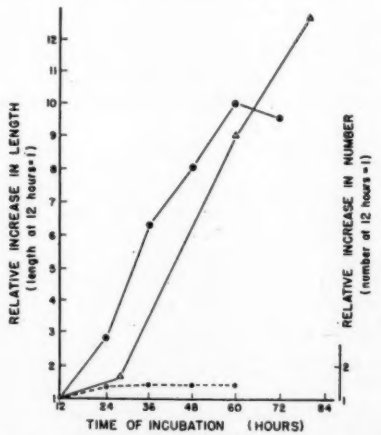

FIGURE 5.—Growth in length of streptomycin-dependent *E. coli* on agar containing no streptomycin. Each point is based on measurements of 50 cells. Circles: cells replated (plate-washing technique) at 12 hours. Triangles: cells not replated. The broken line shows, on the same scale, the increase in cell number determined microscopically, each point representing the average of 200 observations. Inocula from a culture with 25 μg of streptomycin per ml.

were transferred by the washing technique to new plain-agar plates. In this way the microcolonies present at 12 hours were broken up; the single bacteria could be examined microscopically for new cell divisions and easily measured with a *camera lucida*. Figure 5 shows that cell divisions are rare after the first 12 hours of residual growth, whereas cell length, which has remained normal during the first 12 hours, increases enormously. At the same time viability decreases; more and more cells lose the ability to start dividing again when returned to streptomycin medium (fig. 6).

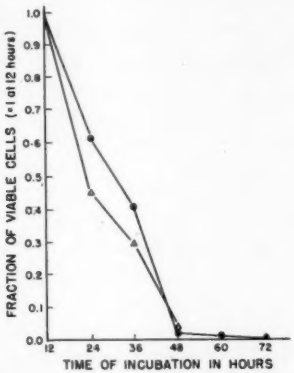

FIGURE 6.—Decrease of viability of "snakes" on agar containing no streptomycin, following the first 12 hours of residual growth. Two experiments. After 12 hours on plain agar, the bacteria were washed, replated on plain agar, incubated further for different periods of time, and then covered with a layer of streptomycin agar, which allowed development of colonies from the cells that were still viable.

BACK-MUTATION IN *Sd-4*

When streptomycin-dependent bacteria are plated on nutrient agar without streptomycin, any nondependent mutants that may be present will divide and give origin to visible colonies. These nondependent mutants may be expected to include two types: sensitive to streptomycin (*R*) and resistant to streptomycin (*RS*). Both types were found. They were classified by picking the mutant colonies and streaking on plain nutrient agar and on streptomycin agar (50 or more μg of streptomycin per ml); only the *RS* types are able to grow on streptomycin agar. In many experiments, the colonies were first transferred to tubes containing 2 ml of plain nutrient broth, incubated for 48 hours, and then tested by streaking.

Contaminants might be scored as mutants. This would result in a negligible error when the number of mutants per plate was high, but in a serious error when the number was low. To reduce this source of error, the strain *Sd-4/1,3,4,5* was used in many experiments, and the mutant colonies were tested by cross-streaking against phages *T2* and *T3* (or *T4*). Those that were either not sensitive to *T2* or not resistant to *T3* (or *T4*) were discarded as con-

TABLE 1

Frequency of nondependent mutants among dependent cells grown in 25 μg of streptomycin per ml and plated on plain nutrient agar. Fourteen experiments.

No. of plates	Mutants per plate Av. ±S.E.	Bacteria plated per plate ($\times 10^7$)	Mutants per 10^8 cells plated
10	12.60 ±1.11	3.8	33.2
15	6.07 ±0.66	2.6	23.4
8	6.63 ±0.96	2.3	28.5
9	6.22 ±0.64	2.7	23.0
7	10.00 ±1.72	2.1	47.6
8	7.50 ±1.09	4.0	18.8
7	11.00 ±1.95	3.8	28.9
8	11.50 ±0.80	3.0	38.3
8	15.88 ±0.92	2.6	61.1
9	11.89 ±1.64	2.9	41.0
10	11.70 ±1.26	3.0	39.0
9	6.56 ±0.60	2.4	27.3
7	13.86 ±3.20	2.4	57.8
9	10.00 ±1.65	2.7	37.0

taminants. They never represented more than a small proportion of the total colonies.

Time of appearance of mutant colonies. No mutant colonies were visible on the plain-agar plates before 8 to 12 hours of incubation; most colonies became visible between 24 and 72 hours of incubation; no new mutant colonies appeared after 7 days of incubation (fig. 7). The total counts of mutants after 7 days' incubation are given in table 1. Figure 7 also shows that the resistant mutants appeared later than the sensitive ones, with an average delay of about 24 hours. The final ratio of *RS* type to *R* type among the total number of scored mutants did not vary much from experiment to experiment (table 2), the average ratio being 0.29 *RS* to 0.71 *R*.

The pattern of appearance of mutant colonies is essentially the result of two factors: the time of occurrence of mutations and the rate of growth of the mutant colonies.

Time of occurrence of back-mutations. If a large part of the mutations detected in the manner described above occurred before plating, that is, if the

TABLE 2

Ratio of resistant to sensitive among nondependent mutants.

	Experiment					Total	%
	a	b	c	d	e		
Sensitive mutants *(R)*	114	2	21	50	42	229	71.1
Resistant mutants *(RS)*	41	5	5	20	22	93	28.9
Total	155	7	26	70	64	322	

Chi-square for homogeneity = 8.75, P = 0.07
= 2.48, P = 0.50, eliminating Experiment b.

mutant colonies stemmed from nondependent cells present as such in the inoculum, this would complicate the use of the method for detecting mutations induced by a mutagenic agent applied immediately before plating on plain agar. The following considerations, however, show that only a small proportion of the total nondependent colonies may be due to mutations that occurred before plating.

Sensitive mutants that may arise during the growth of the dependent bacteria in the presence of streptomycin have no chance of surviving if the concentration of streptomycin is higher than 3 μg per ml. Resistant mutants, on

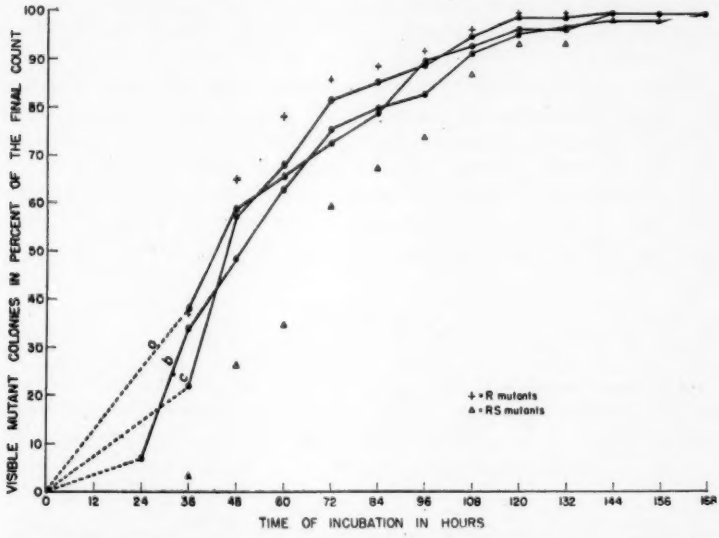

FIGURE 7.—Time of appearance of mutant colonies among streptomycin-dependent bacteria (less than 3.8×10^7 cells per plate) grown to 25 μg of streptomycin per ml and plated on plain nutrient agar. The percentages are calculated from the total number of mutants (29, 70 and 126 for experiments *a*, *b* and *c*, respectively). Triangles: same for *RS* mutants alone (results of the three experiments pooled). Crosses: same for *R* mutants alone.

the other hand, may survive, increase in number, and appear as nondependent colonies on plain agar. Although this possibility cannot be ruled out completely, there is indirect evidence against it. (a) The ratio of *RS* to *R* mutants does not vary greatly from experiment to experiment, as might be expected if this were so. (b) Strain *Sd-4*, serially subcultured (large inocula) for as many as twelve times, gave, when plated on plain agar, the same number of mutants as at the beginning of the experiment. This rules out the possibility of selection in favor of *RS* mutants against the *Sd* cells in the presence of streptomycin. (c) Several *RS* mutants proved to be at a disadvantage when mixed with *Sd* cells in the presence of streptomycin.

Since snakes are polynucleate, they may contribute greatly to the total number of mutations, if mutations occur and are expressed in these cells. Ultraviolet irradiation of *Sd* cells plated on plain agar and incubated for 12 hours increases the number of mutations as compared with non-irradiated controls. No statistically significant increase is detectable when the irradiation is done after 24 or more hours of incubation.

We conclude, therefore, that most mutations occur after plating, during the residual cell divisions, although these data do not exclude the possibility that a few also occur and are expressed after the completion of the residual divisions, in the very first stages of snake formation.

Development of mutant colonies. The generation time of different mutant strains, measured nephelometrically in nutrient broth, was 29, 29, 35, 42 and 54 minutes for five *R* strains, and 29, 33 and 37 minutes for three *RS* strains. The generation time of mutant colonies growing on the plain-agar plates on which they are detected must be considerably slower, in order to account for the shape of the curves of figure 7. Assuming that most of the mutations occur in the first 12 hours of incubation, an approximate estimate of the generation time of the mutant cells during this period can be obtained from the curve of residual growth on agar (fig. 3, triangles), the average number of mutant *colonies* per plate (table 1), and the average number of mutant *cells* per plate after 12 hours of incubation (table 3; the 1 : 100 dilution factor must be

TABLE 3

Number of nondependent cells of mutational origin present among Sd bacteria (grown in 25 μg of streptomycin per ml) plated on plain agar plates and incubated 12 and 14 hours respectively. The plates were "washed" either at 12 or at 14 hours of incubation, and samples of the suspension were replated on a number of plain agar plates; a 1 : 100 dilution is involved in this procedure.

	Washed at 12 hours					Washed at 14 hours				
Exp.	No. of mutant colonies per plate (replating) Av.±S.E.	No. of plates (replating)	No. of *R* mutants	No. of *RS* mutants	*RS* (%)	No. of mutant colonies per plate (replating) Av.±S.E.	No. of plates (replating)	No. of *R* mutants	No. of *RS* mutants	*RS* (%)
A	1.10 ± 0.31	10	....	....		2.70 ± 0.63	10	27	0	
	1.70 ± 0.42	10	13	4	39.3	5.70 ± 0.67	10	55	2	6.7
	2.20 ± 0.53	10	14	8		3.60 ± 0.78	10	30	6	
	2.20 ± 0.53	10	10	12						
B	1.11 ± 0.51	9	7	2		4.67 ± 0.82	9	35	7	
	0.70 ± 0.21	10	5	2	33.4	3.30 ± 0.47	10	25	8	16.3
	0.56 ± 0.24	9	2	3		23.20 ± 2.00	10	196	35	
C	0.57 ± 0.30	7	0	3		4.50 ± 0.99	6	20	5	
	1.44 ± 0.47	9	0	12	27.1	0.67 ± 0.37	9	4	1	24.6
	2.88 ± 0.91	8	19	1		1.89 ± 0.31	9	13	4	
	2.67 ± 0.71	9	24	0		3.44 ± 0.53	9	21	9	
Av.	1.56				33.3	5.37 (3.39)*				15.3

* Calculated disregarding item 23.20 of Exp. B.

taken into account in comparing with data of table 1). An independent estimate of the average generation time of the mutants can be made from the increase in number of mutant *cells* per plate between 12 and 14 hours of incubation (table 3). The two separate estimates are consistent; the generation time of mutated cells, in the period of time considered, is somewhat less than two hours. This means either that the crowding on the plates is already critical (which seems unlikely in the first hours of incubation, because all experiments reported above were done with not more than 3×10^7 cells per plate; compare fig. 4), or that the transition of the mutated cells from the slow division rate typical of the dependent cells in residual growth to the normal division rate of the nondependent cells (*i.e.*, the adjustment of the mutated cells to the new gene-cytoplasm-environment balance) is not a rapid process.

To explain the difference between the curves for *R* and *RS* mutants in figure 7, one must assume either that the transition period is longer for *RS* cells or that such cells are hindered to a greater extent than *R* cells by the presence of dependent bacteria. As a matter of fact, a comparison of the ratios of sensitive to resistant cells after 12 and 14 hours of incubation (table 3) shows that resistant cells under these conditions divide on the average more slowly than sensitive ones. Unfortunately, the type of experiment supplying the data for table 3 does not allow any simple statistical treatment. Additional evidence was obtained from measurements of the diameters of mutant colonies after 168 hours of incubation; on the average, *RS* colonies were always smaller than the *R* ones that appeared at the same time (table 4).

Factors influencing the total mutant count. As the majority of the back-mutations occur during residual growth, one expects that any factor that increases the extent of the latter will also increase the number of mutants. One such factor is the concentration of streptomycin in which the *Sd* cells used as inoculum have been grown (see fig. 3). Table 5 shows the results for concentrations of streptomycin between 10 and 50 μg per ml. For lower concentrations (2 to 6 μg per ml), the mutant frequency ranges between 1.5 and 3.6 per 10^8 bacteria plated.

From the observations on residual growth reported in figure 4, one might expect an increase in frequency of mutants when more than 5×10^7 bacteria

TABLE 4

Size of mutant colonies, classified according to the time of their appearance. All measurements made after 168 hours of incubation.

Mutant type		Time of appearance (hours of incubation)							
		36	48	60	72	84	96	108	120
R	No. of colonies	28	34	11	7	4	1	4	3
	Average diameter	14.3	11.1	8.8	7.5	5.5	4.5	3.4	3.0
RS	No. of colonies		10	3	10	1	3	4	2
	Average diameter		8.5	5.7	6.8	4.0	3.3	2.9	3.3

TABLE 5

Effect of streptomycin concentration in the culture from which the inoculum was taken on the frequency of mutants per bacteria plated. The number of cells plated was always between 1.6 and 2.8 × 10^7 per plate.

Exp.	10 μg/ml			25 μg/ml			50 μg/ml		
	No. of plates	Total no. of mutants	Mutants per 10^8 bacteria plated	No. of plates	Total no. of mutants	Mutants per 10^8 bacteria plated	No. of plates	Total no. of mutants	Mutants per 10^8 bacteria plated
A	9	50	22.6	7	97	57.0	8	174	87.5
B	10	37	22.5	9	70	36.4	8	109	74.3
C	9	68	28.6	9	90	37.4	9	181	72.5

are plated per plate. On the contrary, the extent of the residual growth under these conditions is such as to hinder the development of mutant colonies; the total mutant counts are lower than when fewer bacteria are plated. This holds for bacteria grown in 25 μg per ml of broth. With bacteria grown in 10 μg of streptomycin per ml, differences in frequency of mutants due to different degrees of crowding are much smaller; and with lower streptomycin concentrations they may be completely negligible. These effects of crowding must be carefully considered when one plates large inocula—for example, in experiments using mutagenic agents that kill a high percentage of the bacteria treated.

Estimate of rate of mutation to nondependence. Counts of mutants, expressed up to this point as the number of mutant colonies per 10^8 plated bacteria (mutant frequency), include mostly mutants originating during the residual cell divisions, plus a few that may have been carried over with the inoculum, and possibly some originating in young snake forms. If the number of residual divisions is taken into account, it is possible to estimate a mutation rate per cell per generation that will be exaggerated because mutations in snakes and carried over mutants will be included. The numbers of residual cell divisions for bacteria grown at 10 and 25 μg of streptomycin per ml are given in figure 3. The average mutant frequencies (calculated from data of table 5 and table 1) are 24.5 and 36.1 per 10^8, respectively. Calculations give the following values for mutation rate per cell per division: 4.0 and 4.4 per 10^8, respectively. These are maximum estimates, being based on *viable,* not *total,* cell counts (fig. 3).

The method proposed by LURIA and DELBRÜCK (1943), based on the distribution of mutants over a large number of cultures started from a small inoculum, was used to reach an independent estimate of the rate of mutation to nondependence. *Sd* bacteria were grown with 1 μg/ml of streptomycin, at which concentration sensitive mutants are not killed and dependent bacteria can still grow normally. One experiment of this type (sixty 0.2-ml cultures inoculated with 300 *Sd* cells, grown to turbidity and plated on plain agar) gave an estimate of mutation rate of 1.4 per 10^8 per cell per generation, as calculated from the frequency of cultures with no mutants. Of the 15 inde-

pendent mutants found in this experiment, 4 were resistant to streptomycin and 11 were sensitive.

OBSERVATIONS ON OTHER DEPENDENT STRAINS

A few observations were made on streptomycin-dependent strains other than *Sd-4*.

The residual growth of strain *Sd-3* was studied both in liquid culture and on agar. No striking differences from the behavior of *Sd-4* were observed. Growth in length also occurs to the same extent as in *Sd-4*.

Strain *Sd-26*, although it has the same streptomycin requirements for optimal growth as *Sd-4*, shows a more extensive residual growth when plated on plain agar. Residual cell divisions occur as long as 24 hours after plating, and after 48 hours 14 percent of the cells present at 12 hours are still able to recover if streptomycin is added. Only a few cells develop into snakes. An increase in number of mutants can be produced by irradiating with ultraviolet after as long as 36 hours of incubation on plain agar. Determinations of the frequency of mutants for this strain (grown on 25 μg of streptomycin per ml; 2.1 to 3.4×10^7 bacteria per plate) give an average value of 27.3 per 10^8 plated bacteria. The ratio of *RS* to *R* types among mutants in this strain is completely different from that typical for *Sd-4:* among a total of 124 mutants tested, 86 percent were found to be resistant to streptomycin.

Observations were made on two other dependent strains, isolated from a resistant strain in which dependent mutants were easy to select on streptomycin agar, because of their ability to form faster-growing colonies than the resistant cells. Both these strains, when grown in 25 μg of streptomycin per ml, showed fewer residual cell divisions on agar than *Sd-4*. Filamentous forms were very short, or absent, and mutant frequencies were low (0.34 per 10^8 in one strain, 1.8 per 10^8 in the other). One of the strains, analyzed for ratio of *RS* to *R* among the mutants, gave 74 percent *RS*.

DISCUSSION

The method described above permits selection and scoring of cells that undergo mutation from streptomycin dependence to nondependence, even when the frequency of mutations is as low as 1 in 10^8 cells plated. Increases of mutation frequency induced by some chemical or physical treatment of the bacteria can be easily detected.

Other types of transformations, which also have the character of true mutations but occur with much higher frequency, have been used as tests for induced mutation (see, for instance, KAPLAN 1947, 1949). In these cases, the methods involve no selection, but simple scoring of the mutated and nonmutated types. Until more is known about these high-rate transformations, however, it does not seem advisable to use them as routine tests for induced mutation, in the same way that it would not be advisable to use highly mutable loci in higher organisms for the same purpose. Furthermore, these high-frequency transformations may present problems in scoring of colonies of

alternative types, as every colony is expected to be a mixture of cells of the different types.

More strictly comparable with the method described in this paper is the test for induced mutation based on bacterial resistance to phage (DEMEREC and LATARJET 1946; WITKIN 1947; BEALE 1948). Here the selection is effected either by plating bacteria on agar previously coated with phage, or by spraying phage on bacteria previously plated and incubated for a certain period of time to allow a known number of cell divisions to take place after the mutagenic treatment. The first technique does not reveal *all* the induced mutations, whereas the spraying technique is too laborious a procedure to be used routinely. The method based on streptomycin dependence offers the advantages of the spraying technique, allowing control of the residual growth by simply varying the concentration of streptomycin in which the dependent bacteria are grown, and at the same time is technically much simpler than the method based on phage resistance. The two methods, involving mutation to phage resistance and back-mutation from streptomycin dependence, may also be used together in a double test employing a streptomycin-dependent phage-sensitive strain, because phage can multiply normally on streptomycin-dependent bacteria.

Residual growth is common to many dependent strains of bacteria when transferred to media lacking the required substance; strains dependent on other substances, therefore, might be used in tests for induced mutation. The streptomycin-dependence method has the advantage that streptomycin acts as a selective agent against back-mutants to sensitivity and, as pointed out before, against most of the resistant nondependent mutants. This would not happen with substances that are not antibiotic.

The few observations made on dependent strains other than *Sd-4* demonstrate the possibility of adjusting the test to particular needs by using strains that do or do not form snakes, strains that have low or high spontaneous mutation rates, and so forth.

The problem of the nature of the back-mutations—that is, whether they are gene mutations at the same locus involved in mutation to dependence, or mutations of modifier genes—cannot be elucidated with the strain of *E. coli* used in these studies. This problem has recently been investigated by NEWCOMBE and NYHOLM (1949) and DEMEREC (1950) with strain K-12, which exhibits genetic recombination (LEDERBERG and TATUM 1946).

SUMMARY

When mutant strains of *Escherichia coli* that require streptomycin for growth are transferred to a medium containing no streptomycin, they are able to continue growing for a limited period before they die. This residual growth consists in a number of residual cell divisions, followed by an increase in length of the cells (nuclear division without cell division). The pattern of residual growth is different for different dependent strains. The extent of residual growth is mainly a function of the concentration of streptomycin in which the dependent strains have previously been cultured. During residual

growth on plain nutrient agar, nondependent back-mutants either sensitive or resistant to streptomycin have a chance to form colonies, which can be scored and used to calculate mutation rate. The method described can be used as a very simple and rapid test for induced mutation.

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GENETIC CHANGES WITHIN POPULATIONS AFTER X-IRRADIATION *

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NATURAL populations of cross fertilizing, diploid organisms contain a wealth of concealed genetic variability which arises continuously through recessive gene mutation and is limited in extent by natural selection. The limiting, or equilibrium, frequency of each variant exists when the rate of elimination of the variant equals its rate of production.

The information available at present concerning the genetics of populations has been accumulated through analyses of populations of long standing, which are usually at or near equilibrium conditions. Very little is known of changes that occur during the approach to equilibrium, although predictions, based on mathematical models, have been made. In addition, the effects of differing rates of mutation on neither the equilibria obtained nor the approaches to these equilibria have been studied experimentally. This latter fact hampers geneticists in postulating the effects of atomic and other radiations on human populations.

This report concerns the changes that have been observed in the frequencies of recessive gene mutations in populations of *Drosophila melanogaster* which were exposed to acute X-ray treatments. Although these populations are still being studied, only the data collected during the first 40 weeks will be discussed at this time. Experiments are in progress in which populations are being continuously exposed to gamma-rays; but these are of relatively recent origin and will not be discussed here.

MATERIALS AND METHODS

The populations used in the studies to be discussed were composed of flies derived from the Oregon-R strain of *D. melanogaster*. The derivation of these flies and the composition of the populations will be considered below.

The cages in which the populations were maintained are lucite boxes similar to those used by DOBZHANSKY (WRIGHT and DOBZHANSKY 1946). The cage, which resembles half of an angel food cake, is semi-circular in shape, 4″ high, 2½″ wide, and 23″ and 18″ in outside and inside diameters. In the floor of the cage are 16 holes for the insertion of food cups; 15 of these cups support the population while the 16th is used solely for obtaining egg samples. Since a fresh cup of food is inserted every day from Monday through Friday, each cup remains in the cage for three weeks—one week longer than is required for most of the adult flies to emerge and join the

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population. These cages support populations of approximately 10,000 flies. (A more detailed description is given in WALLACE 1949.)

The populations were kept at 25° ± 1°C, and all genetic tests were made at this temperature.

In studying these populations we limited ourselves principally to the second chromosome, a large V-shaped chromosome which comprises approximately two fifths of the entire chromosomal material of *D. melanogaster*. The series of matings used to derive the original flies and to test individual chromosomes merits detailed consideration (fig. 1).

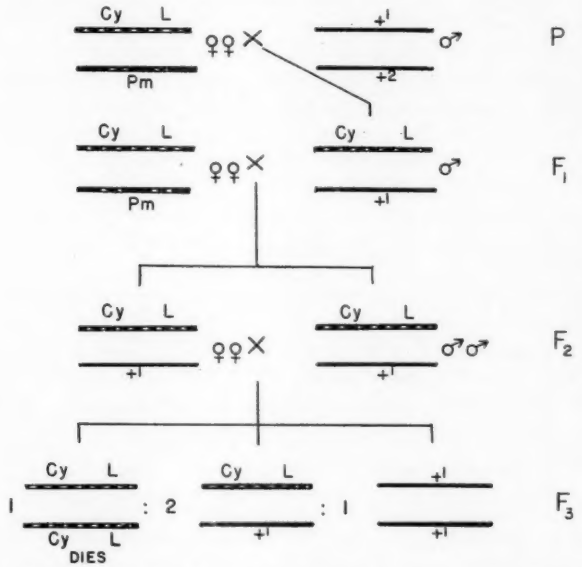

FIGURE 1.—Mating system for the detection of recessive, second-chromosome gene mutations.

The tester stock used in these matings is *In (2L + 2R) Cy, al*^{2} *Cy lt*^{3} *L*^{4}*sp*^{2}/*Pm*. The *Cy L* chromosome carries *Cy* (Curly), a dominant gene that causes the wings to curl upward, and *L* (Lobe), a dominant that imparts lobed or notched shapes to the eyes. In addition to these dominant marker genes, this chromosome carries two large inversions, one in each arm, which effectively eliminate crossing over. Crossing over between the two inversions gives rise to *Cy* flies and to *L* flies; this occurs with a frequency less than one percent. The *Pm* (Plum) chromosome is merely a convenient chromosome against which to balance *Cy L*.

The purpose of the matings is to obtain flies that are homozygous for a given second chromosome. The single *Cy L*/+ F_1 male (see fig. 1) that is re-

mated with *Cy L/Pm* females possesses only one " wild type " second chromosome; consequently, all *Cy L/+* F_2 flies (males and females) carry this chromosome. When the *Cy L/+* F_2 males and females are mated, the F_3 wild flies obtained have two identical second chromosomes. Each gene on the chromosome, therefore, is carried in the homozygous condition. If a recessive lethal is present, there are no wild flies in the culture; if a semilethal or subvital is present, the expected 2:1 ratio is distorted by smaller ratios of +/+ flies; if a visible is present, each wild fly is modified accordingly.

In referring to this series of matings subsequently we shall use " standard matings," " standard test," or some other general term. The test requires seven or eight weeks from the time of the egg sample until the F_3 culture has hatched completely. With few exceptions, three counts are made of each F_3 culture. If fewer than 100 flies are obtained, *Cy L/+* F_3 flies are mated to give an F_4 culture, whose count is combined with that of the F_3.

In order to analyze the populations, samples of eggs were originally removed every two weeks; later, every four or six weeks. Each sample consisted of several subsamples taken on successive days; the randomness of the sample was increased by this subdivision. The eggs were apportioned among several culture bottles so that the larvae developed with a minimum of competition.

The experiments with acute radiations included three populations: population 1 received a high dose (♂ ♂ 7125 r, ♀ ♀ 1012.5 r) of X-radiation; population 2, a low dose (♂ ♂ and ♀ ♀ 1012.5 r); and population 3, the control, none. X-ray treatments were given with a Norelco Searchray machine (North American Philips Co., Inc.) on July 23, 1949. Details of the treatment were as follows:

Target distance	15 cm
Filters	.13 mm cu and .85 mm al
Kilovolts	140
Milliamps	8
Intensity	125 r per minute

During the treatment the flies (males and virgin females) were kept in small gelatin capsules arranged in a single layer over the bottom of a ten centimeter Petri dish. The dish was revolved during treatment. The temperature was not controlled, but was not excessively high at any time.

THE ORIGINAL FLIES

Experimental populations that are to yield results of a general nature must fulfill two requirements; their genetic compositions must be similar but, in order to avoid results that are applicable only to a single genetic situation, each must be genetically heterogeneous. The populations of these experiments, moreover, had to contain second chromosomes devoid of lethals and semilethals.

To obtain populations that met these requirements, a number of males from a mass culture of Oregon-R flies were mated singly with *Cy L/Pm* females, and, in passing through the standard matings outlined above, five *Cy L/+* F_1

males from each of these matings were mated again with *Cy L/Pm* females. From the ratio of *Cy L+* to +/+ in the F_3 it was possible to pick cultures whose chromosomes carried no lethals or drastic viability modifiers. One such culture was chosen from each of sixteen of the original males, and these flies were the source of the populations. To obtain the parental flies required for the three populations, +/+ flies (F_3) were inbred one more generation. The sixteen homozygous stocks are maintained for use in other experiments.

The initial compositions of the three populations are given in table 1. Inspection of this table shows that the postulated requirements were fulfilled, that is, the populations were uniformly heterogeneous.

TABLE 1

Data on the derivation of 16 stocks of flies and their frequencies in populations 1, 2, and 3.

Stock	% wild flies in F_3 cultures	Total number of F_3 flies	% of total population		
			1	2	3
15	33.3	102	2.4	2.3	2.3
43	36.1	368	8.6	8.0	8.0
81	28.8	118	4.3	3.8	3.8
93	29.9	268	5.9	5.4	5.4
114	32.7	330	7.9	7.3	7.2
123	33.6	423	5.7	8.1	8.0
131	36.2	323	7.8	10.0	9.7
143	32.8	183	1.9	1.8	1.7
151	34.1	85	2.8	2.6	2.6
173	37.5	293	8.6	7.9	8.9
184	38.4	86	3.1	2.9	2.8
192	34.8	322	10.2	9.9	9.7
205	27.1	118	3.2	2.9	2.9
263	31.6	313	8.3	8.2	8.7
281	33.3	249	8.3	8.7	8.5
295	30.7	267	11.0	10.3	9.8

During the derivation of these stocks it was noted that Lobe has a variable penetrance; late-hatching flies from crowded cultures frequently have normal appearing eyes and, consequently, the apparent number of *Cy* crossover types is increased. Because of this and because Curly sometimes overlaps wild type, the ratio of wild flies to the total in the culture is more reliable as a measure of viability than the ratio of wild to *Cy L/+* flies; therefore, percentages of wild type flies are given in terms of the total number of flies in a given culture. (Curly and Lobe flies from those cultures that give seemingly high numbers of crossovers, 9 or more, are test-mated with *Cy L/Pm*. To date, none of these tests has indicated an actual loss of *Cy* or *L* from the tester chromosome, but rather a lack of penetrance and a misclassification.)

RECESSIVE GENE MUTATIONS

The parental flies, because of the mating system used for their derivation, were originally free of second-chromosome lethal, semilethal, drastic subvital, and visible gene mutations. To determine the effect of the X-irradiation (or,

in the control, of spontaneous mutation) on the frequencies of such recessive genes, a number of chromosomes were tested periodically by means of the standard test crosses. Each wild type male (P_1) hatching from an egg removed from the population during the egg sampling initiated a succession of matings, which gave information concerning one second chromosome.

Lethals and semilethals. These two classes of mutant genes were indicated by a low frequency of wild type flies in the F_3 cultures of the test series. If any of those cultures contained fewer than 3.1 percent wild flies, that chromosome was considered lethal; if fewer than 15.8 percent, semilethal.

Before examining the data obtained by sampling the populations, two factors will be considered: the effect of culture conditions on the viability of the wild type flies, and the relative viabilities of the two sexes. DOBZHANSKY and SPASSKY (1944) have shown that temperature and other environmental factors can drastically alter the viability of flies homozygous for certain chromosomes.

To test the reliability of our culturing methods, two sets of *Cy L/+* (F_2) parents were used to start two parallel test cultures (F_3) of the first (all populations) and the second (populations 1 and 3) samples. The coefficient of correlation, *r*, of frequencies of wild type flies in 473 of these paired cultures is .877; the probability that this does not differ from r = 0 is negligible. Included in these tests were 43 absolute lethals, which gave no wild flies in either culture, and 2 chromosomes that behaved as absolute lethals in one culture but allowed a few wild flies (less than five percent) to survive in the other.

During the same period data were gathered on the frequency of wild flies of the two sexes. The coefficient of correlation for the two sexes was .860. Forty six lethals in this series of tests acted as lethals in both sexes, one behaved as an absolute lethal for females but allowed a few (less than five percent) of the males to survive. The difference between the values of *r* for cultures and for sexes is not significant.

A summary of the data on lethals and semilethals is given in table 2. The total number of flies counted to obtain these data was 1,470,686. In connection with this table, several explanations are necessary. The decrease of "average culture size" from 300 or more flies to about 170 that occurred after the first or second sample reflects the change from two parallel cultures per chromosome to a single culture. Associated with this change to single cultures and, later, with the termination of population 2, was an increase in the number of chromosomes tested per sample. In sample 12 of populations 1 and 2 and sample 13 of population 3 several cultures were classified as "normal" without an actual count. At that time it had been decided to make full counts only on lethal and semilethal chromosomes; but it soon developed that counts were necessary in order to make a proper decision and so full counts were immediately resumed. In calculating the frequency of semilethals in a sample, only non-lethal chromosomes were considered.

TABLE 2

Summarized results of chromosome analyses.

Population	Sample	Average culture size	No. lethal	No. semi-lethal	No. normal	Total chrom.	% lethal	% lethal adjusted	% semi-lethal
1	1	316.8	24	4	103	131	18.3	18.0	3.7
	2	315.4	22	5	129	156	14.1	13.4	3.7
	3	170.0	20	7	146	173	11.6	10.2	4.6
	4	180.9	18	3	157	178	10.1	8.4	1.9
	6	157.3	22	10	170	202	10.9	8.5	5.6
	8	170.2	34	10	204	248	13.7	10.6	4.7
	10	176.8	33	12	181	226	14.6	10.9	6.2
	12	175.8*	42	21	226*	289	14.5	10.1	8.5
	14	186.7	50	26	291	367	13.6	8.5	8.2
	16	170.9	73	33	283	389	18.8	13.0	10.4
	18	159.9	75	39	288	402	18.7	12.2	11.9
	20	200.5	34	17	195	246	13.8	6.7	8.0
2	1	330.9	7	1	144	152	4.6		0.7
	2	159.3	14	....	150	164	8.5		0
	3	186.2	7	2	159	168	4.2		1.2
	4	189.2	12	2	172	186	6.5		1.2
	6	166.1	8	1	179	188	4.3		0.6
	8	201.6	12	4	221	237	5.1		1.8
	10	167.6	16	7	203	226	7.1		3.3
	12	182.2**	25	2	246**	273	9.2		0.8
3	1	315.6	1	....	132	133	0.8		0
	2	289.6	....	....	52	52	0		0
	3	159.6	4	....	179	183	2.2		0
	5	153.6	3	....	209	212	1.4		0
	7	194.6	12	3	248	263	4.6		1.2
	9	169.6	9	1	275	285	3.2		0.4
	11	180.6	14	2	267	283	4.9		0.7
	13	191.8**	20	4	361**	385	5.2		1.1
	15	185.0	15	8	354	377	4.0		2.2
	17	158.6	25	8	375	408	6.1		2.1
	19	157.1	20	6	383	409	4.9		1.5
	22	189.5	21	5	263	289	7.3		1.9

* 2 "normal" cultures not counted.
** 1 "normal" culture not counted.

From the data on lethals given in table 2 (see fig. 2) it is obvious that their frequencies in the populations did not remain constant. In the control population the lethals increased in frequency until, in sample 22, more than seven percent of the chromosomes tested were of this type. The line of best fit for these data is $Y = .0034X$ (Y = frequency of lethals; X = sample number); there is no justification for computing any curve other than this straight line at present. The increase of 0.34 percent lethals per sample was the increase with respect to the sample interval of two weeks; the increase per generation was probably somewhat greater. Under the crowded conditions of the population cage, 12 or 13 days elapse from egg to adult; the generation time which also depends upon the time of reproductive activity of the adults, might easily be five or seven days longer than the two weeks between samples.

The lethals in population 1 underwent more complicated changes in frequency than those of population 3. During the first four samples there was an orderly decrease in the frequency of observed lethals (from 18.3 percent to 10.1 percent); subsequently there was an increase at a rate approximating that of the control. (A preliminary report of these early samples has been published; WALLACE 1950.)

The lethals observed in the irradiated populations represent the sum of induced and spontaneous lethals. The best estimate of the frequency of induced lethals in the various samples of these populations is the difference between the observed frequency and the expected spontaneous frequency (0.34 percent × sample number). The adjusted frequencies for population 1 (dotted line in fig. 2) drop rapidly for four samples and then remain approxi-

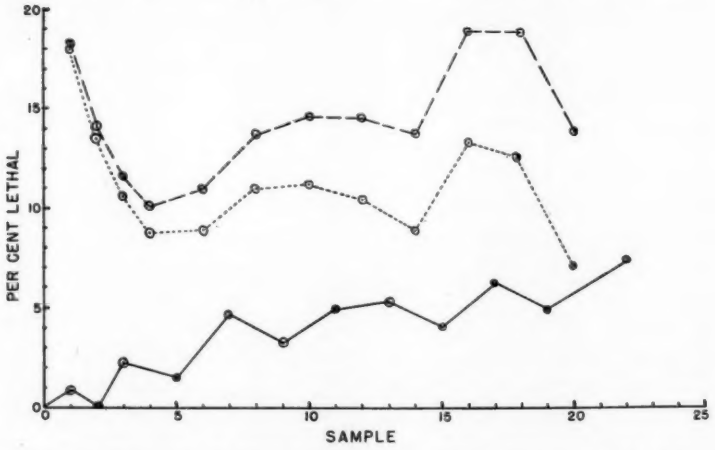

FIGURE 2.—Frequency of lethals in chromosome samples from populations 1 and 3. —— —— —— total observed lethals in population 1, - - - - - - calculated induced lethals in population 1, ———— observed lethals in population 3.

mately constant, with an average frequency of about ten percent. This ten percent represents those lethals which are being eliminated from the population very slowly, if at all. The other portion of the original lethals (eight percent) must have been eliminated through a selective disadvantage of heterozygous individuals. (The amount of homozygosity for individual, randomly distributed, lethal genes, negligible at these frequencies, is approximately F^2/n where F is the frequency of lethal chromosomes and n is the number of potentially lethal loci. In the first generation of population 1, assuming 400 loci as a minimum, no more than 0.008 percent lethal homozygotes were formed.)

An estimate of the decrease in the adaptive value of the eliminated heterozygotes may be obtained by subtracting the constant ten percent from the ad-

justed frequencies. This second adjustment leaves us with values of 8 percent, 3^+ percent, and 0^+ percent in the first three samples; an elimination of 0.5 per generation could well account for this decrease. It is tempting to equate this elimination with the 50 percent reduction in fertility which is characteristic of translocation heterozygotes and which, in a population, could explain the selective disadvantage of the eliminated lethals. Information pertinent to this correlation is available (LEA 1947; PATTERSON, STONE, BEDICHEK and SUCHE 1934) and can be summarized as follows: (1) After treatment of *D. melanogaster* males with 7000 r, 55 percent of the viable sperm carry gross aberrations (LEA). (2) About 50 percent of these gross aberrations are translocations (LEA). (3) The second chromosome is involved in approximately 85 percent of all translocations (PATTERSON). (4) Slightly more than 80 percent of all second chromosome translocations are lethal when homozygous (PATTERSON). (5) About 19 percent (the product of the above frequencies) of the sperm of males irradiated with 7000 r carry second chromosomes that are involved in lethal-translocation complexes. (6) Since males contribute only half the second chromosomes in population 1, the original frequency of lethal-translocation complexes in the second chromosomes of population 1 was about 9 to 10 percent. This value agrees with the 8 percent that was indicated by the lethal data given above; it may be assumed that the rapid elimination of lethals from population 1 was the result of an association of some lethals with translocations.

To recapitulate briefly, the available data indicate that one-half of the original induced lethals in population 1 were associated with translocations and, through the reduction of fertility in translocation heterozygotes, were eliminated from the population; the remainder persisted at a relatively constant frequency through twenty samples, and therefore must have minor, if any deleterious effects in the heterozygous individuals. Superimposed on the changes in the frequency of induced lethals there was a constant accumulation of spontaneous lethals.

The data obtained from population 2 (table 2) were consistent with the conclusions outlined above but, because of the low frequencies of the lethals, were noninformative; this population was discontinued after the 12th sample.

Table 2 gives data that have been obtained on the frequencies of semilethals in the populations. These will not be discussed, except to point out that semilethals increased in frequency in all populations and that this increase was more rapid in population 1 than in population 3.

Viability modifiers. In addition to those genes that drastically reduce the viability of homozygous individuals, there are genes (subvitals) that lower viability only slightly. To isolate and detect individual chromosomes that carry subvitals requires examination of a large number of F_3 flies; the smaller the degree of subvitality, the more laborious is the task of demonstrating it. In the present analyses it was important to have data on many chromosomes, and consequently the data on each were limited. However, it

is possible to examine the available data and describe in general terms changes that occurred in the populations.

In a typical sample the tested chromosomes give, in the F_3 cultures, an array of percentages of wild flies ranging from 0 percent to 40 percent or more. It is possible to calculate for this array an "average viability," which can serve as a measure of the entire range of viabilities observed. It is also possible, ignoring lethals and semilethals, which fall well outside the range of sampling error of chromosomes with normal viability, to compute an "average viability" of "normal" chromosomes. These two averages can be used to demonstrate changes that occurred within the populations, to indicate the relative effects of genes with major and minor deleterious effects on viability within populations, or to contrast changes which occurred in different populations. It should be noted that the average viability of *all* chromosomes shows a negative correlation with the frequency of lethal chromosomes, since individual lethal cultures lower the average perceptibly. (The averages given in table 3 and figure 3 are averages of *frequencies* of wild flies in the tested cultures.)

The regression lines ($\hat{Y} = a + bX$) for the average viabilities (table 4) draw attention to several points: (1) If all chromosomes are considered, irradiation lowered the average viability of flies homozygous for the various chromosomes found within the treated populations. (The $\hat{Y}$ values of the first

TABLE 3

The average frequency of wild-type flies in all chromosomes tested and in the "normal" chromosomes.

Sample	Population 1		Population 2		Population 3	
	All	Normal	All	Normal	All	Normal
1	24.41	30.66	30.12	31.74	31.83	32.08
2	26.71	31.94	28.96	31.64	31.65	31.65
3	26.61	31.11	29.66	31.25	30.77	31.46
4	26.97	30.39	29.04	31.30	....	....
5	....	....	....	....	31.05	31.49
6	26.44	30.79	30.51	31.98	....	....
7	....	....	....	....	30.44	32.14
8	24.80	29.63	29.26	31.21	....	....
9	....	....	....	....	30.54	31.64
10	24.53	29.85	29.40	32.36	....	....
11	....	....	....	....	29.69	31.40
12	24.75	30.81	29.38	32.55	....	....
13	....	....	*	*	29.95	31.79
14	24.52	29.99			....	....
15	....	....			29.20	30.84
16	22.64	29.90			....	....
17	....	....			28.57	30.87
18	21.95	29.26			....	....
19	....	....			30.37	32.25
20	24.81	30.33			....	....
22	....	....			30.04	32.77

* Terminated.

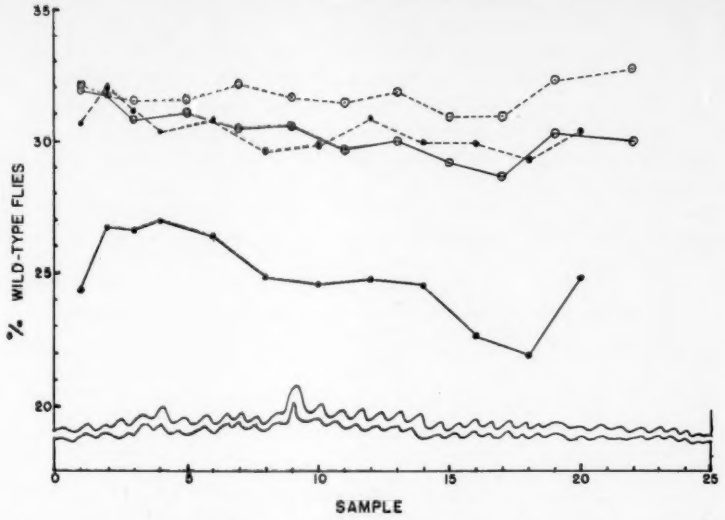

FIGURE 3.—Average viability of flies homozygous for chromosomes sampled from populations 1 and 3. ● population 1, ○ population 3, ———— average of *all* chromosomes, – – – – – average of *normal* chromosomes.

samples of the three populations are significantly different.) (2) The initial average viability of flies homozygous for normal chromosomes was not significantly lowered by irradiation, although, taking the data at face value, the average was lower in irradiated populations and lowest in the population which received the most severe treatment. (3) Considering all chromosomes again, the average viabilities decreased significantly as the populations aged. This decrease reflects the accumulation of lethals that have been considered above. Population 2, which showed no significant change, was probably in the process of losing the few lethals that were associated with translocations

TABLE 4

Regressions ($\hat{Y} = a + bX$) of the average viability of all chromosomes of each sample and of "normal" chromosomes. s = the standard error of b. p = the probability that β is 0. Regression of all chromosomes of population 1, sample 4 and later, is shown as 1a.

	Population	Regression	s	p
all	1	$Y = .2656 - .0017X$	.0005	<.01
	1a	$Y = .2658 - .0022X$	.0006	<.01
	2	$Y = .2964 - .0002X$	.0006	>.50
	3	$Y = .3138 - .0010X$	.0003	<.01
"normal"	1	$Y = .3107 - .0007X$	.0003	.02–.05
	2	$Y = .3128 + .0008X$	.0004	.05–.10
	3	$Y = .3160 + .0001X$	.0003	>.50

while accumulating spontaneous lethals; the net effect of the exchange was so slight that conveniently sized samples failed to demonstrate any significant change. (4) A significant decrease occurred in the average viability of normal chromosomes of population 1; the apparent increases in viability occurring in the other populations were not significant. (To indicate that population 1 had a period of elimination of lethals and a period of accumulation, table 4 includes a regression of the average viability of all chromosomes calculated for sample 4 and later.)

The mean viabilities of flies carrying tested normal chromosomes do not adequately describe the genetic situations within the populations. It is con-

TABLE 5

The variance (s^2) and coefficient of skewness (s_k) of the distribution of viabilities of "normal" chromosomes.

Sample	Population 1		Population 2		Population 3	
	s^2	s_k	s^2	s_k	s^2	s_k
1	1.943	−.49	1.646	.26	1.590	.28
2	1.524	−.21	1.523	−.17	.975	.09
3	1.684	−.28	1.269	.09	1.447	−.21
4	1.671	−.34	1.545	−.03	....	....
5	....	....	....	....	1.577	.00
6	2.018	−.09	1.641	−.05	....	....
7	....	....	....	....	1.569	.18
8	2.259	.01	1.895	.08	....	....
9	....	....	....	....	1.518	.05
10	2.379	.14	1.706	.00	....	....
11	....	....	....	....	1.733	.02
12	2.648	−.02	2.355	.09	....	....
13	....	....	*	*	2.083	.20
14	2.774	−.11			....	....
15	....	....			1.771	.05
16	2.622	.24			....	....
17	....	....			1.936	.01
18	2.447	.03			....	....
19	....	....			1.701	.03
20	2.864	.12			....	....
21	....	....			....	....
22	....	....			2.601	.01

* Terminated.

ceivable, for instance, that the average remained constant in a given population as a result of an equilibrium between deleterious and beneficial genes. A process of this sort, however, should increase the variance of the array of viabilities shown by the tested chromosomes. Since the array of any sample was not necessarily normal in distribution, the coefficient of skewness may also indicate alterations in genetic composition. The variances and coefficients of skewness were calculated for the normal chromosomes of our samples, and are given in table 5 and figures 4 and 5. The calculations are based on the standard measure distributions which were constructed by the use of $\bar{p}$, the average viability of the sample, p, the frequency of wild flies in each F_3 cul-

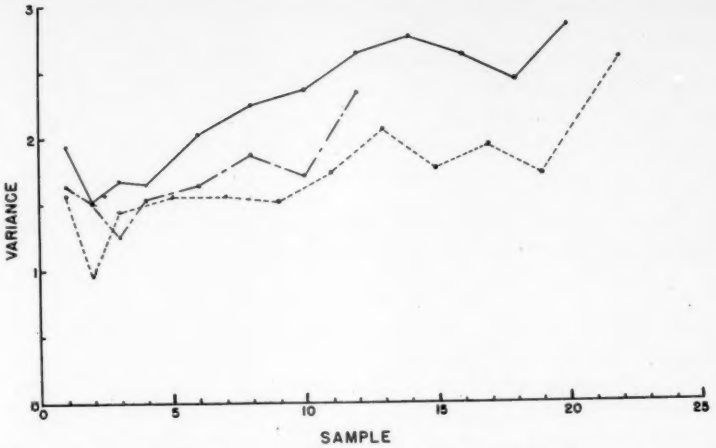

FIGURE 4.—Variances of the distributions of viabilities of flies homozygous for normal chromosomes from the experimental populations. ———— population 1, —— – —— population 2, – – – – – population 3.

ture, and $\sqrt{pq/n}$ where n is the number of flies in the tested culture. This distribution equalizes the variations expected from cultures of different sizes and, if all normal chromosomes were identical, would result in a normal curve with a variance of 1 and a coefficient of skewness of 0. (To simplify the calculations, s_k was calculated as 3 (mean—median)/σ; this has a standard

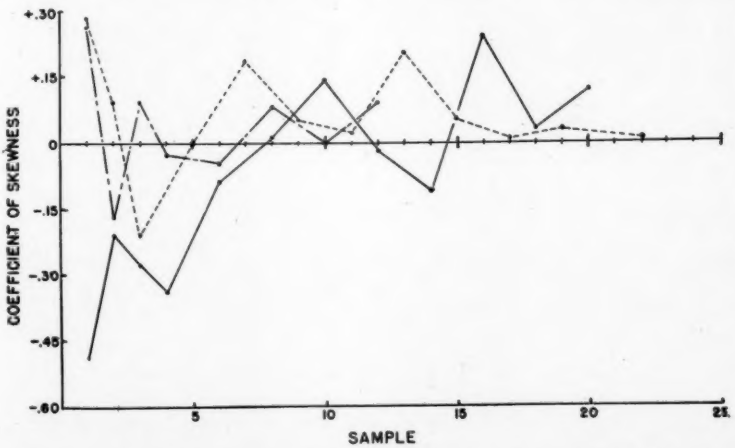

FIGURE 5.—Coefficient of skewness of the distributions of viabilities of flies homozygous for normal chromosomes from experimental populations. ———— population 1, —— – —— population 2, – – – – – population 3.

error of $\sqrt{3/N}$ where N is the number of normal chromosomes in the sample.)

It is evident from the data in table 5 and from figure 4 that the variances of the distributions of viabilities of normal chromosomes increased steadily in all populations, including the control. The regressions of these variances are given in table 6. The increases observed in each population were significant, but the differences in rates of increase in the three populations were not significant. The populations form a series, however, with population 1 having the greatest variance and the most rapid increase and population 3 having the smallest variance and the least rapid increase.

TABLE 6

Regressions ($\hat{Y} = a + bX$) of variance (s^2) and coefficient of skewness (s_k) of all populations. s = standard error of b. p = the probability that β is 0.

	Population	Regression	s	p
s^2	1	$Y = 1.632 + .064X$	.010	<.01
	2	$Y = 1.333 + .064X$	.024	.02–.05
	3	$Y = 1.253 + .044X$	.019	.01–.02
s_k	1	$Y = -.33 + .026X$	.006	<.01
	2	$Y = .03 + .000X$	.013	>.50
	3	$Y = .10 - .004X$	.004	.3–.4

Significant changes in the coefficient of skewness occurred in population 1 (tables 5 and 6 and figure 5). In the early samples, the distribution of normal chromosomes of this population was definitely skewed to the left—an indication that many of the normal chromosomes were actually subvital. This skewness decreased steadily until there was no essential difference between population 1 and the control. No significant changes occurred in the skewness of population 2 and 3. The regression of the coefficients of skewness in population 1 may well be greater than indicated in the table, for this value was calculated for all samples from the population, whereas it may be that there was no change in s_k since sample 10.

These analyses reveal changes that took place within the normal chromosomes of the populations. In the control, despite a significant increase in variance, the mean viability and the coefficient of skewness remained constant. This may be interpreted as a balance, probably temporary, between gene mutation to subvitals and mutation to supervitals (or selection for superior chromosomes among either the original sixteen or their recombinants). These changes occurred without disturbing the symmetry of the distribution. In population 1 the average viability showed a small but significant decline, the variance increased, and skewness, originally markedly to the left, was lost. (It should be recalled that the variance was computed in each case by the use of $\bar{p}$ of that sample; hence, the increase in variance is not a result of the shift in the mean.) Again, the increase in variance indicates an increase in the relative array of viabilities, but, in this case, with an excessive shift to the left which lowered the mean. The decrease in the mean and the simultaneous

loss of negative skewness seems to result from the maintenance of a constant number of superior chromosomes which, as the mean shifted, appeared in the upper tail of the standard measure distributions of the later samples. A small rise in the average viability between the first and second samples of population 1 may indicate that a number of the subvitals of this population were associated with translocations and were quickly eliminated from the population.

In the above discussions, attention was called to several items—some significant and others not—which, considered together, cannot be ignored. First, the secondary increase of lethals in population 1 occurred at a slightly (not significantly) greater rate than that at which lethals accumulated in population 3. Second, the semilethals increased in all populations but, again, the increase was more rapid in population 1 than in the control. Third, the average viability of normal chromosomes of population 1 showed a significant decrease, whereas that of population 3 did not. Fourth, the variance of viabilities of normal chromosomes increased in all populations but this increase was at a more rapid rate in population 1 than in population 3. It seems that the factors followed in our studies changed with a greater rapidity in the irradiated than in the control population. Two mechanisms might explain this phenomenon: either the current rate of spontaneous mutation of the treated population is higher than that of the control, or the changes that occurred in the populations resulted both from mutation and from recombination and the number of possible recombinants was greater in the treated population. The greater variety of recombinants in this population would result from the great variety of induced mutant genes.

Sex-linked lethals. No extended effort was made in these populations to determine frequencies of sex-linked lethals. Theoretically, these lethals should not accumulate to any great extent in any population. If a population contains an initial frequency greater than the equilibrium frequency ($3 \times$ mutation rate), this frequency should rapidly approach equilibrium. At a time corresponding to sample 21, virgin females were collected from egg samples of population 1 and 3; one X-chromosome from each female was tested for lethality. Among 323 chromosomes tested from population 1, 5 lethals were recovered (1.5 percent); among 306 tested from population 3, 3 lethals were found (1.0 percent). The difference between the populations was not significant. Estimating that 1000 r induce 3 percent sex-linked lethals, the original frequency of lethals in population 1 was probably 10 percent to 12 percent; there can be no doubt that this frequency decreased during the untested interval.

DISCUSSION

In spite of the fact that these populations have existed for more than a year, this account is still of the nature of a progress report. Consequently, a full discussion at this time is impossible and any discussion must be somewhat speculative.

As a general statement, it may be said that no genetic changes occurred in the irradiated populations that require explanations based on phenomena other than those already familiar to geneticists. This applies specifically to the fate of the recessive lethals in population 1. There are certain aspects that require close scrutiny but these cannot be considered unique to X-rayed populations. It has been suggested, for instance, that recombination may be playing a role in the formation of lethals and semilethals found within population 1. DOBZHANSKY (1946) has described "synthetic lethals," lethals which seemingly arise through crossing over rather than gene mutation in *D. pseudoobscura*. IVES (1945), too, has reported that a lethal second chromosome taken from a population of *D. melanogaster* proved to be a combination of a semilethal and a visible gene mutation. Unfortunately, there is no reason to expect that combinations of genes which have lethal effects when homozygous will have detectable effects on viability when isolated from one another.

The relative roles of minor and major viability modifiers in determining the changes that occur in the average viability of homozygous individuals is shown clearly in tables 3 and 4. In population 3, mutations that only slightly alter the viability of homozygous flies have yet to alter the average viability of normal chromosomes, whereas the accumulated lethals have manifested themselves clearly in this respect.

It may not be out of place to emphasize here that the well-being of a population as such depends only partially on the characteristics of homozygous individuals; a population of any appreciable size consists almost entirely of heterozygous individuals. The adaptive value of a population must be evaluated by techniques other than the one used here.

Interest in this report will be centered largely in the bearing these studies have on human populations exposed to atomic radiations. In discussing the results in this connection it must be remembered that the experimental fly populations are young, as populations go, and that the present conclusions based on them are tentative. Furthermore, the irradiated populations of these experiments were artificial, in the sense that only non-lethal second chromosomes were originally present. The final result of their irradiation as indicated by the available recessive-lethal data for population 1, seems to be merely a shift toward the final equilibrium frequency. Assuming that these experimental populations will prove to be similar to those studied by IVES (1945), the final frequency of lethal chromosomes in both irradiated and control populations will be approximately 50 percent; the X-rayed population should be the first to approach this equilibrium.

If a population were at equilibrium at the time of irradiation, the frequency of lethals would be raised above the equilibrium value. After irradiation those lethals that were associated with translocations would be rapidly eliminated, just as they were in these studies. A much slower decrease in lethal frequency would then take place as the equilibrium value became reestablished. We have no evidence to indicate that in this final adjustment there would be an appreciable discrimination between the newly induced lethals and the

original, "natural" lethals; there merely would be an elimination of lethals in the population until equilibrium existed once more.

The tremendous variability that exists in the gene content of the various chromosomes of natural populations makes it seem doubtful that the different possibilities of recombination would be greatly increased by the additional mutations induced by radiations. The effect of recombination is suspected of being greater in population 1 than in population 3 because the original chromosomes were isolated from an extremely small population (mass culture) and, although they probably differed somewhat, there was probably a great deal of uniformity at many gene loci.

To predict events that might occur in human populations from data of these and similar Drosophila populations, one must bear in mind the differing biologies of the two groups. As an example, the fate of translocations may be considered. In a population of flies, the perpetuation of a chromosome in the population depends in part upon the fertility of individuals which carry the chromosome. As a result of meiotic disturbances, both male and female flies heterozygous for translocations produce many gametes that form inviable zygotes. This semi-sterility resulted in a systematic reduction in the frequency of the translocated chromosomes in population 1. In human populations, where the actual number of offspring per family is seldom as large as the maximum possible and is always much lower than the number of eggs produced, this semi-sterility might not have as marked a selective effect against the translocations as the degree of semi-sterility might indicate.

Human values, notably absent in Drosophila populations, must be considered, too, in any transfer of conclusions from the present type of experiments to situations involving human beings. MULLER (1950) has pointed out that in the history of any population each deleterious gene must eventually cause the death of an individual ("genetic death"). According to MULLER, if a deleterious gene is induced in a human population through man-made radiations, the death of an individual will result and the responsibility for this death will rest on the human society. The proper evaluation of moral issues of this sort cannot be based upon experimentation.

SUMMARY

Genetic analyses have been made of populations of *Drosophila melanogaster* exposed to single X-ray treatments. The parental males of population 1 received 7125 r; females of population 1 and males and females of population 2 received 1012.5 r; the flies of population 3 were untreated. Before treatment, all flies carried second chromosomes that were free of lethals and drastic subvitals.

The recessive lethals of population 1 decreased from an original 18.3 percent to 10.1 percent in approximately four generations, and then increased at a rate comparable to that of the control. The early elimination of lethals from population 1 indicated a selective disadvantage of heterozygotes of 0.5, and was adequately explained by lethal-translocation association.

Analyses of lethals, semi-lethals, average viabilities of flies homozygous for "normal" chromosomes, and variances of the array of "normal" viabilities indicate that changes occurred at a more rapid rate in population 1 than in the control. It has been suggested that this was the result of a greater number of possible gene combinations in the irradiated population and that gene mutation was supplemented by gene recombination.

The discussion touches briefly on general problems of irradiated populations.

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AN ANALYSIS OF SEEDLING MUTANTS (SPONTANEOUS, ATOMIC BOMB-RADIATION-, AND X RAY-INDUCED) IN BARLEY AND DURUM WHEAT[1]

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A GENE mutation may be defined as a change in or at a single gene locus, although in practice it may be difficult to distinguish such mutations from grosser changes involving segments of chromosomes containing more than one gene. Such gene mutations arise spontaneously, or they may be artificially induced by heat or chemical treatments, aging, or various kinds of ionizing radiations. Most mutations in the strict sense should be transmitted in approximately normal, theoretical ratios. On the other hand, grosser changes, involving chromosomal aberrations, should tend to modify the transmission of affected chromosomes—resulting in abnormal ratios of genes associated with them.

The present studies involve seedling mutant characters of barley and durum wheat. Most of the mutants were induced by X ray and atomic bomb irradiation, but in barley a small number of mutants of spontaneous origin was available for comparison.

The observations make possible further comparisons between the genetic effects of X radiation and of atomic bomb-induced radiation, and to some extent, between mutants from these two sources of irradiation and spontaneously-occurring mutants. Also a considerable body of information was obtained on segregation ratios of individual mutants as well as different types of mutants, which throws some light on whether or not the mutations behaved like gene or "point" mutations.

REVIEW OF LITERATURE

STADLER (1930) reported that about 90 percent of X ray-induced mutations in barley were found in the seedling stage, and were chiefly chlorophyll abnormalities. Most of these mutant characters were lethal or unfavorable to growth. The proportion of mutant plants was less than 25 percent in the segregating head progenies of the X_2* generation, and the proportion of heterozygous plants was less than 50 percent in the same generation. In later generations, segregation ratios approached normal.

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* Plants grown from x-rayed seeds are referred to as the X_1. Progenies of these plants are the X_2 generation; the next generation is the X_3, etc.

GUSTAFSSON (1938, 1941, 1946, 1947), in his extensive X radiation experiments on barley, obtained results similar to those of STADLER (1930). He noted that some of the lines had a deficiency, and some had an excess of recessives. It was postulated (1941) that the aberrant segregations resulted from gametic elimination.

The reader is referred to the previous reports by SMITH (1950) for the results of earlier studies on the mutants used in the present tests. This publication also cited several reports on the biological effects of the Test Able atomic bomb. The evidence indicated that atomic bomb-induced radiations and X rays produce genetic changes that are essentially identical as to type. Observations on the X_3 generation of these mutants were presented in somewhat greater detail by MOH (1950).

The reader is also referred to the review paper by SMITH (1951) for further literature on genetic, irradiation, and other studies with barley. In general it seems that mutants in barley, both induced and spontaneous, behave as simple recessives, although there have been a few exceptions.

MATERIAL AND METHODS

The present study involved tests on the X_3 and X_4 generations of X ray-induced and atomic bomb-induced mutants in Trebi barley and Carleton durum wheat. Some spontaneous mutants were available in the barley for comparison with the radiation-induced mutants.

Samples of 100 seeds were taken from X_2 plants which had been shown by previous greenhouse tests to be heterozygous for a mutation. These seed samples were planted in rows which were two feet long and two inches apart. Similar mutants were planted together for convenience of comparisons.

When the seedlings were about one cm in height, observations were recorded, especially on those mutants most likely to change in appearance. Notes were taken at intervals of one to three days until the seedlings were about 12 cm tall (about the time the second leaf appeared). Then the number of mutant seedlings, and the total number of seedlings in a row (plant progeny) were counted.

The X_4 tests were much the same as described above for the X_3, except that the samples did not all contain 100 seeds. This was due to the fact that some weak or partially sterile plants were tested.

The number of independent mutants tested in X_3 was less than in X_4, because, unless an X_2 plant had enough seeds for a 100-seed greenhouse planting in addition to the number needed for a planting in the field for later generation studies, the mutant was not scored in X_3.

RESULTS OF X_3 AND X_4 SEEDLING PROGENY TESTS

Types of seedling mutant characters

Classification of the seedling mutants into specific types was arbitrary, because many mutants were intermediate between two types, and some varied in intensity of leaf color under different environmental conditions or at different ages. As a matter of fact, it can be said that there is an almost con-

tinuous series of mutant chlorophyll colors, and any grouping of them must be arbitrary. SMITH (1950) classified the seedling mutants of barley and durum wheat into six major types: White, yellow-green, yellow, virescent, shrivel, and miscellaneous. These mutant types were retained in the present study.

Relation of percentage of recessives to origin of mutants and types of mutants

A total of 397 independent mutants represented by 650 heterozygous plants were tested for the proportions of normal and mutant progeny in X_3 (table 1). Of these 397 mutants, 294 were bomb-induced; 89 were X ray-induced; and 14 were of spontaneous origin. A total of 581 independent mutants represented by 5,653 heterozygous plants were tested for segregation ratios in X_4 (table 1). Of these 581 mutants, 436 were bomb-induced; 127 were X ray-induced; and 18 were of spontaneous origin.

TABLE 1

Percentage of mutant offspring in X_3 and X_4 segregating progenies induced by atomic bomb and X-rays irradiation and from spontaneous mutation in barley and durum wheat.

Material	Source of mutation	No. of independent mutants tested		Total plants tested		Percentage of recessives			
						Range[1]		Mean[2]	
		X_3	X_4	X_3	X_4	X_3	X_4	X_3	X_4
Barley	Bomb	232	355	360	3538	1-49	1-60	21	21
	X-ray	85	119	138	1103	2-30	3-27	19	20
	Spontaneous	14	18	22	185	13-29	13-26	23	20
	Total	331	492	520	4826			20	20
Durum wheat	Bomb	62	81	123	770	2-36	3-45	20	20
	X-ray	4	8	7	57		9-24	21	19
	Total	66	89	130	827			20	20

[1] The range indicates the wide differences between certain segregation ratios.
[2] Of indicated number of independent mutants, not of individual plants.

The statistical computations on differences between mutants of bomb, X-ray, and spontaneous origin; between different types of mutants; and between X_3 and X_4 populations were not made. It was evident that, although some of the differences might be shown to be statistically significant, they were not great enough (in most cases less than two percent) to be of any apparent biological importance—except possibly in the dwarf white group of mutants in barley. The dwarf whites, differed from the others by being transmitted in rather consistently low percentages, about 10 percent, as compared with about 20 percent for the other types (table 1). There is no apparent explanation as to why this type of mutant was transmitted in low proportions, unless it was just a case of small populations.

TABLE 2

Percentage of seedling emergence in X_3 progeny tests of plants heterozygous for mutants from the three sources of mutation.

Material	Source of mutant	Percentage of emergence				Total number
		60–69	70–79	80–89	90–100	
Barley	Bomb	2	12	63	283	360
	X-ray		5	16	117	138
	Spontaneous			1	21	22
Durum wheat	Bomb		2	11	110	123
	X-ray				7	7
Total		2	19	91	538	650
Percent		0.3	2.9	14.0	82.8	

Relation of percentage of recessives to seed germination

Of the 650 heterozygous plants tested in X_3 more than 95 percent gave higher than 80 percent germination (table 2). There were 19 of the 650 plants that gave 70–79 percent of seedling emergence. This might be due to the unfavorable growing conditions in the greenhouse, as shown by the fact that three plants, with enough seeds for duplicate plantings, germinated more than 90 percent in the second planting as compared with 70–79 percent in a previous planting. Seeds of only two plants had lower than 70 percent germination. Unfortunately there was insufficient seed of these two plants for duplicate tests. Therefore, failure of seed germination was probably not an important factor in the segregation ratios as a whole, although it was in the case of certain individual mutants. For this reason, the data on the percentages of seedling emergence in the X_4 progenies are not presented here.

TABLE 3

A grouping of mutants on the basis of the percentage of recessive progeny in X_3 and X_4 populations.

Material	Source of mutant	Percentage of recessives						Total number of mutants	
		X_3			X_4				
		<16%	16–34%	>34%	<16%	16–34%	>34%	X_3	X_4
Barley	Bomb	49	178	5	65	284	6	232	355
	X-ray	21	64		28	90	1	85	119
	Spontaneous	2	12		5	13		14	18
	Total mutants	72	254	5	98	387	7	331	492
	Percent	21.8	76.7	1.5	19.9	78.7	1.4		
Durum wheat	Bomb	18	43	1	17	63	1	62	81
	X-ray		4		3	5		4	8
	Total mutants	18	47	1	20	68	1	66	89
	Percent	27.3	71.2	1.5	22.5	76.4	1.1		

Proportion of mutants which segregated in high or low frequencies

Theoretically, a single recessive gene change would give a ratio of 3 normal to 1 mutant seedling in the progeny of a heterozygous plant, *i.e.*, 25 percent of mutants. In table 3 mutants segregating in the range from 16 to 34 percent are not rejected by the χ^2 test for goodness of fit to the 3 : 1 ratio based on 100-seedling populations. There was a considerable number of the mutants tested which did not fall in the range of 16–34 percent. In barley there were 77 of 331 mutants (23.3 percent) with segragation ratios rejected by the χ^2 test for goodness of fit to the 3 : 1 ratio in X_3, and 105 of 492 mutants (21.3 percent) were rejected in X_4. In durum wheat there were 19 of 66 mutants (28.8 percent) with segregation ratios rejected by the χ^2 test for goodness of fit to the 3 : 1 ratio in X_3, and 21 of 89 mutants tested (23.6 percent) were rejected in X_4. Possible explanations for these results will be considered in the discussion.

TABLE 4

A selected group of bomb-induced mutants which segregated in distinctly high frequencies.

1949 mutant progeny number	Type of mutant	Average percentage of recessives in X_3	Percentage of recessives in X_4		No. of plants tested in X_4	No. of heterozygous plants
			Average	Range[1]		
903.2 to .4	Yellow	41	38	8–50	24	23
903.355	Yellow-green	....	60	56–63	5	5
903.814 to 819	Twisted leaf	....	40	18–55	33	33
903.820 to 825	White	49	51	29–62	43	43
Total					105	104

[1] The range indicates the wide differences between certain segregation ratios.

On the basis of a single recessive factor segregation, the χ^2 value for the test of significance of difference between the observed and expected numbers of heterozygous plants = 49.5. The P value is much less than 0.01.

Percentage of recessives in a selected group of barley mutants which segregated in distinctly high or low frequencies

Data from 38 of the 105 barley mutants which segregated less than 16, or more than 34, percent recessive progeny in X_4 were selected for presentation in more detail. These 38 selected mutants were more or less consistent in segregating high or low frequencies of recessives among their progenies. Results of tests with four mutants which segregated a high percentage of recessives in X_3 and X_4 are presented in table 4. Results of tests with 34 mutants which segregated a low percentage of recessives in X_3 and X_4 are presented in table 5. The number of heterozygous X_3 plants deviated significantly from the theoretical 66.7 percent in both the high and low frequency groups. Likewise, the proportion of recessive progeny in segregating populations deviated significantly from the theoretical 25 percent. These facts and other points in connection with these mutants which segregated in clearly aberrant ratios, will be considered in more detail in the discussion.

TABLE 5

A selected group of mutants which segregated in distinctly low frequencies.

1949 mutant progeny number*	Type of mutant	Average percentage of mutant offspring in X_3	Percentage of mutant offspring in X_4		No. of plants tested in X_4	No. of heterozygous plants
			Average	Range		
903.1	Yellow	8	7	2-13	15	7
903.59 to .61	White	2	4	1-8	32	18
903.73	White	6	10	1-25	10	6
903.100 to .102	White	7	9	4-16	25	14
903.128	White	....	7	1-15	7	4
903.129 to .130	Yellow-green	....	6	4-10	11	7
903.164 to .165	Yellow	3	3	2-5	15	2
903.201 to .204	White	7	4	2-16	28	14
903.275 to .276	White	....	5	3-6	11	3
903.322 to .324	White	1	3	1-5	26	6
903.327	White	....	6	5-7	6	2
903.336 to .339	Yellow	....	2	1-5	20	13
903.350	White	....	2	1-2	6	2
903.392	Yellow	....	9	3-12	6	5
903.403	White	2	1	1-2	14	3
903.446 to .449	White	5	12	2-23	27	12
903.527	White	....	7	2-17	6	5
903.615	White	10	13	8-18	12	5
903.651	White	....	4	3-4	6	3
903.690	Yellow	....	3	2-3	5	3
903.712	Yellow-green	6	5	1-9	29	16
903.766	Yellow	....	6	4-10	5	3
903.826 to .828	Whitish-green	....	12	6-18	17	13
903.843 to .844	Creamex	....	6	8-13	12	7
903.901	Yellow-green	....	9	8-10	6	2
903.941 to .944	White	....	3	1-8	24	10
902.8	Yellow-green	10	8	4-16	16	9
902.27	Yellow-green	11	5	3-7	13	4
902.63 to .64	White	4	8	2-14	19	10
902.115 to .116	Yellow	....	9	3-15	11	8
902.126 to .128	Yellow-green	....	5	1-10	18	11
902.179 to .180	Yellow	7	6	5-8	10	3
902.211 to .213	White	....	7	2-10	18	9
902.214 to .218	White	10	8	2-18	36	20
Total					522	259

On the basis of a single recessive factor segregation, the χ^2 value for the test of significance of the difference between observed and expected numbers of heterozygous plants = 68.3. The P value is much less than 0.01.

* Mutant numbers of 903 are from bomb irradiation, and of 902 from X-ray irradiation.

DISCUSSION

As stated above, there were no striking differences in the transmission of mutant genes whether they arose spontaneously or were X ray- or atomic bomb-induced. Such differences as were observed may most logically be attributed to small samples, especially in the spontaneous group. However, it would be particularly difficult to obtain a large enough sample of spontaneous mutants.

Although there were no marked differences between mutants from the three sources (bomb, X rays, spontaneous) and between X_3 and X_4, there were decided differences in the segregation ratios of mutants from the same source (cf. tables 1 and 3). This phenomenon will be considered in some detail.

Theoretically, a point mutation would give rise to 25 percent of recessives in a segregating population. Mutant segregates forming less than 16 percent or more than 34 percent of the progeny fall outside two standard deviations from the theoretical 25 percent on the normal curve. These upper and lower limits of segregation ratios were calculated by the χ^2 test based on a 100-plant population. There are a number of possible explanations for such deviations from the expected 3:1 segregation ratios that may be considered: (1) Normal chance variations; (2) duplicate genes (15:1 ratio) and complementary genes (9:7 ratio); (3) low germinability of the X_3 seeds; and (4) unequal transmission of the normal and mutated chromosomes.

The first possibility is based on the statistical point of view. It could happen that some heterozygous plants segregated in simple Mendelian ratios and might by chance give unexpected proportions of recessives. The normal probability is that such cases would constitute less than 5 percent of the total mutants tested. However, there were over 20 percent of the barley mutants which were tested that gave lower than 16 percent or higher than 34 percent of recessive offspring in X_3 and X_4. In durum wheat about 25 percent of the mutants showed similar variations from theoretical ratios. It is extremely unlikely that all these aberrant segregation ratios were due to normally-expected extreme chance deviations.

Considering the second possibility, duplicate factors would yield a 15:1 ratio (6.25 percent of mutant offspring) and complementary factors would yield a 9:7 ratio (43.75 percent of mutant offspring). In diploid species, duplicate genes are not uncommon. In polyploid species, such as durum wheat, it is probable that many segments of chromosomes of a genom, or functions of genes, are duplicated in the other genom. The nature of gene interaction would determine the segregation ratio.

For example, on the basis of Mendelian expectations, if duplicate genes are present, 1 out of 16 plants in the segregating generation would be homozygous for both recessive genes, and 1 out of 4 plants would be heterozygous for both genes. If the mutant character gave a lethal effect (such as a white or a yellow seedling), then there would be a 4/15 phenotypically normal individuals which would transmit the mutant character in 15:1 ratio, and 4/15 phenotypically normal individuals which would transmit the mutant character in 3:1 ratio. If the two genes were complementary, and the mutant character were lethal, there would be 4/9 phenotypically normal individuals which would segregate in a 3:1 ratio, and another 4/9 would segregate in a 9:7 ratio. In any case, duplicate or complementary genes would give characteristic and predictable ratios in succeeding generations. The data in tables 4 and 5 indicate that duplicate or complementary factors are probably not responsible for more than a few if any of the aberrant ratios.

A third possibility is that low germination of certain classes of seeds might be a cause for the aberrant segregations. It was evident in table 2 that the percentage of germination of X_3 seeds was high. Most of the progenies with aberrant ratios had more than 90 percent seedling emergence; all had higher than 80 percent seedling emergence. It seemed, therefore, that failure of seeds to grow could not be considered as the important factor influencing the ratios in this study. This was further borne out by the X_4, although these data were not analyzed in detail for this purpose.

A fourth possibility is that the aberrant segregation ratios could result from unequal transmission of the normal and mutated chromosomes. A mutant gene might have, as a result of pleiotropism, an effect on the competitive ability of the spore receiving it. That effect might be to give the spore an advantage in fertilization—in which case the gene would be transmitted in higher than normal frequency. On the other hand, and more probably, the gene might have a deleterious effect, resulting in a reduced transmission of the affected chromosome. Chromosomal aberrations, such as deficiencies and duplications, have been shown to change the proportion of recessives by modifying the transmission of genes associated with them (cf. SMITH 1948).

If both a genic change and a deficiency were induced in the same chromosome, and the microspores were non-functional because of the deficiency, four kinds of female and two kinds of functional male gametes could be produced—including those resulting from crossing over (see fig. 1). Under these conditions, a reduced transmission of the mutant gene might result. On the other hand, if the genic change and the deficiency were induced in different chromosomes of the same homologous pair, a high proportion of the offspring might be mutants. These two cases are diagrammed in figure 1.

Cases more or less extreme than the two diagrammed in figure 1 could be postulated. Thus, if there were no crossing over between the mutated locus and the deficiency, there would be two kinds of female and only one kind of functional male gamete. In case (a) of figure 1, mutant gene *w* would be eliminated entirely by the deficient male gametes in each generation, and could be transmitted only by the female gametes. Since *W* is dominant to *w*, there would be no possibility of obtaining a homozygous recessive. All the progenies would be phenotypically normal and equally divided among the *WW* and *Ww* genotypes.

Furthermore, in case (b) of figure 1, only the mutant gene *w* would be transmitted by the male gametes, if the dominant gene *W* were completely linked with a deficiency which resulted in non-functional male gametes. Half of the progeny would be mutants, *ww*, and the other half would be phenotypically normal, but all heterozygous *Ww*. Thus, 50 percent recessive offspring would be the maximum that could be obtained. In case of partial ovule sterility caused by deficient female gametes, or in case of non-random segregation of chromosomes, the proportion of mutant progeny could be higher than 50 percent. An interesting point is that all the phenotypically normal individuals would be of the same constitution as the parent—heterozygous for *w* and for the deficiency.

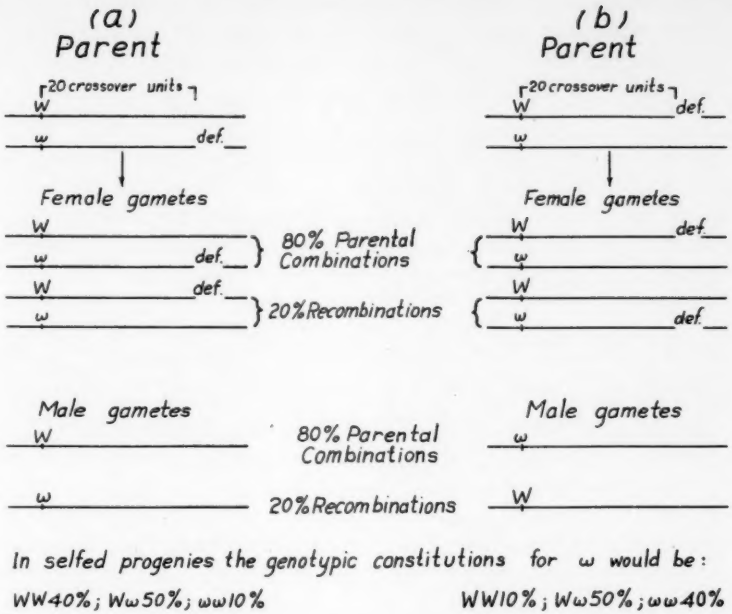

FIGURE 1.—A diagram showing the effect of a deficiency on the transmission of a gene. (a) Assuming that a deficiency and a gene mutation ($W \rightarrow w$) were induced in the same chromosome, and that the deficient male gametes were non-functional, there would be four kinds of functional female gametes and two kinds of male gametes. The proportion of functioning male gametes with mutant gene w would be reduced unless recombination approximated 50 percent. The proportion would be dependent on the crossover value. If there were 20 percent crossing over, as illustrated above, the proportion of W to w would be 4:1 among male gametes, thus giving 10 percent homozygous recessives. (b) If the induced gene mutation and the deficiency were on homologues instead of the same chromosome, the ratio of W to w would be 1:4 among male gametes, giving 40 percent homozygous recessives.

Four mutants which gave a high proportion of heterozygous plants and a high percentage of recessive offspring (cf. table 4) rather closely fit the expectation referred to in the preceding paragraph. Of 105 X_4 plants, 104 were heterozygous and almost all gave a high proportion of recessive progeny. One heterozygous plant gave a low percentage (8.2 percent) of recessive progeny. Another plant was homozygous normal. These two plants may have represented recombinations between the mutated gene and the postulated aberration.

The considerable number of mutants characterized by a low frequency of transmission (data from some of which are presented in table 5) are probably best accounted for on the basis that they are associated with aberrations on the same chromosome. Since there were many more transmitted in low than

in high frequencies, it seems likely that a considerable proportion of them were the result of, or closely associated with, deficiencies or other aberrations, or possibly with pleiotropic effects that reduced the transmission of chromosomes containing the mutant genes.

As pointed out by STADLER (1933), genic changes and certain chromosomal aberrations (deficiencies) cannot always be distinguished. GOODSPEED (1930) found that pink-flowered plants in *Nicotiana tabacum,* which arose as a result of irradiation of carmine-flowered plants, resulted from a deletion involving the chromosome segment containing the carmine gene. Genetically it appeared to be a gene mutation from carmine to pink. A number of mutant characters in Drosophila, such as " Notch " and " Minute," have been shown to be the result of segmental deficiencies. MULLER (1935) found that mutants with a two-gene deficiency (yellow and achaete) arose from crossing over between two inverted X chromosomes in Drosophila. Individuals having those deficiencies were extreme yellow and achaete types. MOHR (1938), and MOHR and MOSSIGE (1942) also reported similar phenomena in Drosophila.

Gametophytes containing an aberrant chromosomal complement are not necessarily non-functional, although the microgametophytes are usually affected more than the corresponding megagametophytes. MCCLINTOCK (1931) demonstrated a number of viable deficiencies in maize, and also found that homozygous deficient tissue in corn could be viable (1938). BURNHAM (1932) noted a plant with a reciprocal interchange which had approximately 25 percent of aborted pollen, instead of the usual 50 percent observed in plants heterozygous for other interchanges. One of the interchanged segments comprised only a few chromomeres of the satellite-chromosome. STADLER (1933) studied a deficiency in maize involving a terminal or nearly terminal segment of Chromosome 10. The deficient chromosome complement was transmitted through the female gametophyte. The complete failure of transmission of deficient chromosome complements through the pollen was probably due to the fact that the second nuclear division was delayed; or to the fact that deficient pollen grains shrivelled quickly, possibly before fertilization was completed.

In the present study X_4 progeny tests have shown that at least in most cases, a low or high percentage of recessive progeny in the X_3 generation was associated with similar results in the X_4. Some of the X_3 plants that gave aberrant ratios were obviously partially sterile. Thus, there is a clue that the transmission of some of the mutant genes may be associated with genic sterility or chromosomal aberrations. However, condensed chromosomes such as those of barley at meiosis are not so favorable for revealing small deficiencies or duplications as are the prophase chromosomes of maize or the salivary gland chromosomes of the Diptera. It is planned, nevertheless, to study further the 38 selected mutants, and a number of others, which gave aberrant ratios, particularly with reference to sterility and cytologically irregularities.

SUMMARY

1. The study involved atomic bomb- and X ray-induced, and spontaneous seedling mutants in barley and durum wheat. Progeny tests were made of 650 X_2 plants, representing 397 independent mutants; and of 5,653 X_3 plants, representing 581 mutants.

2. The mean percentages of recessive offspring in the X_3 progenies of heterozygous plants were: In barley, 1) 21 percent for 232 bomb-induced mutants; 2) 19 percent for 85 X ray-induced mutants; 3) 23 percent for 14 spontaneous mutants. In durum wheat, 4) 20 percent for 62 bomb-induced mutants; 5) 21 percent for 4 X ray-induced mutants. In X_4 progenies the corresponding values were: 1) 21 percent for 355 bomb-induced mutants; 2) 20 percent for 119 X ray-induced mutants; 3) 20 percent for 18 spontaneous mutants; 4) 20 percent for 81 bomb-induced mutants; and 5) 19 percent for 8 X ray-induced mutants, respectively.

3. Thus, the difference between mutants from the bomb, X ray, or spontaneous sources were not large enough to be of any apparent biological importance. There were also no clear differences in the transmission of mutants in the X_3 and X_4 generations. This was generally true for the mutants individually, as well as collectively.

4. There were no distinct differences in the segregation ratios of the six types of mutants (white, yellow-green, etc.)—with the possible exception of the "dwarf white," a sub-group of the white mutants in barley. The mean percentage of dwarf white seedlings in the progeny of heterozygous plants from both bomb- and X-ray-irradiated material was 10 percent. This consistently low transmission of dwarf white mutants may be accounted for by the fact that only a few (10 in X_3 and 13 in X_4) mutants of the type were available for testing. Otherwise there is no ready explanation for the result.

5. More than 20 percent of the mutants tested in X_3 and X_4 segregated in ratios significantly different from the theoretical 3:1. Most of the mutants segregating in aberrant ratios (34 out of 38 which were considered) were associated with a low transmission of the mutated gene. These results were considered in some detail in the discussion.

6. Germination in most of the progeny tests was over 90 percent. Variations in germination did not seem to be associated with the percentage of recessive segregates except with certain individual mutants. Hence, apparently the aberrant segregations were not the result of differential seed germination *per se*.

7. It was concluded that chromosomal aberrations (possibly deficiencies) associated with a considerable portion of the mutations, or pleiotropic effects of the mutant genes, best accounted for the fact that over 20 percent of the mutants tested segregated in ratios that deviated significantly from the theoretical 3:1. The results could readily be accounted for if it were assumed that a considerable portion of the mutations were in fact deficiencies rather than gene or "point" mutations—an hypothesis which is being checked by further tests.

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A DIFFERENCE IN SKELETAL TYPE BETWEEN RECIPROCAL HYBRIDS OF TWO INBRED STRAINS OF MICE (C57 BLK AND C3H) *

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DIFFERENCES between reciprocal hybrids of strains of various animals are not uncommon. In the house mouse an example is the incidence of spontaneous mammary cancer in crosses between " high incidence " and " low incidence " strains (MURRAY and LITTLE 1935). When it may be assumed that the cause of the differences between the reciprocal hybrids is not genetic, as in matings between inbred strains, new possibilities are presented for analyzing non-genetic causes of morphological and physiological variation.

The purpose of this paper is to describe reciprocal crosses between two highly inbred strains of mice, differing in skeletal type, which resulted in F_1 hybrids showing a significant tendency to resemble the skeletal type of the strain of the female parent.

Matings were made, reciprocally, between these two strains for the purposes (1) of determining the nature and estimating the number of pairs of genetic factors necessary to account for the difference in skeletal type between the strains, and (2) of studying the effect, if any, of several environmental variables on a genetically homogeneous, although hybrid, group of F_1 animals. This paper deals with one aspect of the second purpose. The difference in skeletal type between the reciprocal hybrids represents a major finding in the study of the environmental variables. The difference suggests that some elements of the environment, furnished by the ova or by the mothers to the developing embryos, differ in the two strains, and that the nature of these elements may be identified by appropriate experimental techniques, such as for example by transplantation of ova or of ovaries.

STRAINS OF MICE

The two strains of mice (*Mus musculus*) used as the source of parents for this experiment were the Jackson Laboratory strains, C57 blk (non-agouti, *aa*) and C3H (wild type, *AA*). Five sublines were represented among the C57 mice and two among the C3H mice.

From a previous survey of skeletal types in strains of mice (GREEN 1941), it was known that these two inbred strains differ markedly with respect to the type of the axial skeleton. Over 95 percent of the mice in the C57 strain

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have 13 pairs of ribs and 6 lumbar vertebrae (13/6), a few have only 5 lumbar vertebrae (13/5), a few have 5 lumbar vertebrae if viewed from the right side, but 6 if viewed from the left side ($13/_6^5$), and a few have the reverse type of asymmetry ($13/_5^6$). In the C3H strain, these same four types of skeletons occur, but their frequency distribution is different. There are very few of the 13/6, $13/_6^5$ and $13/_5^6$ types, and over 95 percent of the 13/5 type. The percentages of these skeletal types in samples of 395 C57 mice and 967 C3H mice (including those in the earlier survey) are given in table 1.

TABLE 1

Relative frequency of skeletal types in two inbred strains of mice, C57 blk and C3H, and in their reciprocal hybrids. Skeletal type is given as the ratio of thoracic to lumbar vertebrae; $13/_6^5$ and $13/_5^6$ designate mice with asymmetrical sacrums.

Dam	Sire		Offspring					Number
			13/5	$13/_6^5$	$13/_5^6$	13/6	Other	
			percent					
C57 × C57		♂♂	1.1	3.3	1.1	94.5	0.0	183
		♀♀	1.4	0.0	0.0	98.6	0.0	212
		Tot.	1.3	1.5	0.5	96.7	0.0	395
C3H × C3H		♂♂	98.7	0.4	0.4	0.4	0.0	476
		♀♀	94.9	2.8	0.8	1.2	0.2[a]	491
		Tot.	96.8	1.7	0.6	0.8	0.1	967
C57 × C3H (13 matings) (89 litters)		♂♂	33.1	21.3	5.0	39.6	1.0[b]	338
		♀♀	24.0	18.1	5.3	52.3	0.3[c]	304
		Tot.	28.8	19.8	5.1	45.6	0.6	642
C3H × C57 (13 matings) (60 litters)		♂♂	67.8	10.4	4.4	16.9	0.5[d]	183
		♀♀	45.5	13.2	3.0	37.7	0.6[e]	167
		Tot.	57.1	11.7	3.7	26.9	0.6	350

a: unclassified. b: 14/5, 14/6, unclassified. c: 12/6. d: $13/_6^7$. e: 14/5.

Twenty-six matings were made between the C57 and C3H strains. Thirteen of the matings involved C57 ♀♀ × C3H ♂♂; the other thirteen were the reciprocal type, C3H ♀♀ × C57 ♂♂. These matings together produced 992 F_1 mice in 149 litters. Thirty-one matings between pairs of F_1 mice produced 1278 F_2 mice in 152 litters. The skeletal types of all mice were determined by means of a clearing and alizarin staining technique such as described by CUMLEY, CROW and GRIFFIN (1939). Non-breeding animals were prepared for examination between the sixth and thirtieth day of age.

DIFFERENCE IN RECIPROCAL HYBRIDS

Considered as a group, the F_1 animals were intermediate between the two parental strains in the sense that nearly 40 percent were 13/5, nearly 40 percent were 13/6, and the remainder were either $13/_6^5$ or $13/_5^6$. Considered as

two groups, one from C57 mothers, the other from C3H mothers, a difference between the reciprocal hybrids appears, even though each group is still intermediate between the parental strains. Of the 642 hybrid mice raised by C57 mothers, 29 percent were 13/5 and 46 percent were 13/6. In contrast, of the 350 hybrid mice raised by C3H mothers, 57 percent were 13/5, and 27 percent were 13/6. Since the C57 mothers were 13/6 and the C3H mothers were 13/5, the F_1 mice tend to resemble the maternal strain in skeletal type. The frequencies of the four skeletal types with the sexes separated are given in table 1.

A difference between the reciprocal hybrids of inbred strains, present at birth and not confined to the male sex, suggests that some features of the embryonic environment or of the cytoplasm of the ova must be different in the two strains. Before either of these explanations may be accepted, however, it is necessary to establish that the difference between the reciprocal hybrids is not the consequence of the hybrids being non-comparable with respect to other known or possible causes of skeletal variation. Among the other variables possibly associated with skeletal variation are (1) sex, (2) size of litter, (3) age of mother, (4) month or season of birth, and (5) serial number of the litter. Our interest is not to estimate the exact effect of each of these variables on the incidence of the skeletal types, but rather to ascertain that, whatever the effects, they do not contaminate the test of the difference between the reciprocal hybrids.

Sex. In both types of hybrids, as well as in the C57 and C3H strains, males have the smaller number of lumbar vertebrae more frequently than do females. In the mating of C57 ♀ ♀ × C3H ♂ ♂, 33 percent of the males and 24 percent of females were 13/5. The difference, 9 percent, is 2.6 times larger than its own standard error. Equal or greater differences may be expected to arise by chance alone less than once in 100 times on a hypothesis of no difference. In the reciprocal mating, C3H ♀ ♀ × C57 ♂ ♂, 68 percent of the males and 46 percent of the females were 13/5. This difference, 22 percent, is likewise highly significant. The relative frequencies of these and the other skeletal types in the two sexes separately are in table 1.

Two preliminary tests indicate that the difference between the reciprocal hybrids is not a consequence of an unusual sex distribution. First, the ratio of males to females in both types of hybrids is not significantly different from the expected ratio of 1 : 1. In the mating of C57 ♀ ♀ × C3H ♂ ♂, there were 338 males and 304 females. In the reciprocal mating, there were 183 males and 167 females. Second, with males and females considered separately, the dissimilarity of the reciprocal hybrids is still apparent in the totals of table 1. On the hypothesis of no association of type of mating and skeletal type and using the frequencies of the four skeletal types 13/5, $13/_6{}^5$, $13/_5{}^6$, 13/6, we found that for males $\chi^2 = 58.3$ and that for females $\chi^2 = 23.5$. These are both considerably larger than the critical value of χ^2 (0.01, 3) = 11.34. The pattern of the deviations of observed from expected frequencies indicates clearly the tendency for the hybrid offspring to resemble the strain of the female parent in skeletal type.

Litter size. With respect to litter size, the two hybrid groups may not be comparable. The mean litter size for matings of C57 ♀ ♀ × C3H ♂ ♂ was 7.2 and of C3H ♀ ♀ × C57 ♂ ♂ was 5.8. Such a difference may be due to differences in fertility between the pure strain dams, or to differences in viability of the hybrid offspring. It is apparent that if skeletal type is strongly associated with litter size, the apparent difference between the two types of hybrids may be a litter size difference only.

Age of mother, etc. The remaining three variables—age of mother, month of birth, serial number of the litter—are highly correlated and therefore to a large extent are inseparable in this experiment. This is so because all of the matings were made during the month of March, between mice one month of age, and production of litters continued throughout the year and into the early months of the following year. For convenience, we will deal with the serial number of the litter. Because of the high correlation, serial number of the litter actually stands for an amalgamation of influences associated with age of mother and month of birth, as well as with serial number.

The test for a difference in skeletal type between the reciprocal hybrids was performed with consideration for these variables. It is described in the next section. The analysis yields answers to the questions of interest in the following order: (1) are the two kinds of matings different with respect to the skeletal types of the offspring after removing the effects of serial number of litter, litter size, and sex, and (2) are the 26 matings homogeneous with respect to incidence of the skeletal types after removing the effect of type of mating, serial number of litter, litter size, and sex?

THE TEST FOR THE DIFFERENCE

An analysis of variance and covariance is the only general method available for testing a difference between types of matings after adjusting the difference for the effect of several other variables (age of mother, litter size, sex). The method may be used if the data fulfill certain presuppositions about the variables of interest. That is, the variables analyzed should be normally distributed and have uniform variance within the 26 matings. The original records do not fulfill these requirements, as may readily be seen from the following argument. A litter may consist of as many as 8 kinds of mice (2 sexes × 4 skeletal types). We may reduce this number to only 4 kinds of mice: ♂ 13/5, ♂ not 13/5, ♀ 13/5, ♀ not 13/5. But even this simplification, giving the *numbers* of mice of each type, does not yield variables suitable for an analysis of variance. On the contrary, the numbers may be supposed to be distributed as a Poisson variable or at least as components of a Poisson variable, the litter size. This supposition is reasonably fulfilled by the data available. For such variables, the variance is equal to the mean and so does not satisfy the condition of uniform variance when the mean changes.

Several transformations of scale for stabilizing the variance of a Poisson variable have passed into general use. These are based upon the square-root transformation suggested by BARTLETT (1936), but are in general useful only

when the mean expectation is 3 or more. We are indebted to Dr. John W. Tukey for suggesting a transformation which may be used when the mean expectation is as small as 1 and for devising a form of analysis of variance and covariance for adjusting the transformed numbers for the effects of other variables (Tukey 1949). The transformation is $x_a = \sqrt{n_a} + \sqrt{n_a + 1}$, where n_a is the number of mice in a litter of any size showing the characteristic a and where $a = 1$ for ♂ 13/5, $a = 2$ for ♂ not 13/5, $a = 3$ for ♀ 13/5, $a = 4$ for ♀ not 13/5. The size of a litter is $n = n_1 + n_2 + n_3 + n_4$. It is readily seen that when $n_a = 0$, $x_a = 1$, when $n_a = 1$, $x_a = 2.4$, and so on.

Table 2 shows the averages of the x_a's and of the serial numbers m of the litters for each of the 13 matings of the two types. The means of the transformed numbers for each type of mating show that the 13/5 skeletal type is rarer in offspring of C57 ♀ ♀ and more frequent in offspring of C3H ♀ ♀. That is, 2.28 is less than 2.95 and 1.88 is less than 2.33. The question at issue is whether the means for the types of mating will be significantly different *after* the variables have been adjusted for covariation with serial number of the litter (age of mother), size of the litter, and sex.

TABLE 2

Means of the transformed number x_a and of the serial numbers m of the litter for each mating of the two types. Type 1 is C57 ♀♀ × C3H ♂♂, type 2 is C3H ♀♀ × C57 ♂♂. $x_a = \sqrt{n_a} + \sqrt{n_a + 1}$ where n_a is the number of mice in a litter with characteristic a, and where $a = 1$ for ♂ 13/5, $a = 2$ for ♂ not-13/5, $a = 3$ for ♀ 13/5, $a = 4$ for ♀ not-13/5.

Mating number	Type 1. Means of: No. of litters	Males		Females		Serial number m	Type 2. Means of: No. of litters	Males		Females		Serial number m
		13/5 x_1	Other x_2	13/5 x_3	Other x_4			13/5 x_1	Other x_2	13/5 x_3	Other x_4	
1.	9	2.1	3.3	1.9	3.0	5.0	6	2.4	2.6	1.8	2.7	3.5
2.	5	2.9	3.0	1.7	3.5	3.0	5	2.4	2.1	1.6	2.6	3.0
3.	6	2.1	3.5	1.5	3.2	3.5	5	4.0	1.8	2.5	2.7	3.0
4.	7	2.3	2.4	1.4	3.2	4.0	7	3.7	2.1	2.9	2.5	4.0
5.	7	1.4	4.6	1.4	3.9	4.0	8	2.7	2.6	2.2	3.0	4.5
6.	10	1.8	3.9	1.1	4.1	5.5	7	3.4	1.9	1.4	2.7	4.0
7.	8	3.2	2.4	3.6	2.7	4.5	2	3.4	2.0	3.3	2.4	1.5
8.	2	2.4	4.5	1.0	3.4	1.5	2	4.9	1.0	2.3	1.7	1.5
9.	7	2.3	3.4	1.9	2.8	4.0	3	2.9	1.5	2.6	1.5	2.0
10.	7	2.8	3.5	1.5	3.4	4.0	3	2.2	2.4	3.0	1.5	2.0
11.	7	2.4	2.6	2.0	2.8	4.0	4	3.6	1.4	3.4	2.0	2.5
12.	7	3.0	3.1	2.4	3.3	4.0	4	2.7	2.4	2.1	3.5	2.5
13.	7	1.4	3.1	2.3	2.3	4.0	4	2.6	2.4	2.7	2.6	2.5
Type means	89	2.28	3.26	1.88	3.28	4.17	60	2.95	2.10	2.33	2.53	3.18
Grand means	149	2.55	2.80	2.06	2.98	3.77						

TABLE 3

Analysis of variance of transformed numbers x_α, adjusted for covariance with serial number of litter, and analysis of variance of skeletal type numbers, adjusted for covariance with litter size and sex.

Source	df	A. Mean squares and products adjusted for serial number				B. Mean squares and products adjusted and rotated					C. Mean squares, completely adjusted	F	F(0.05)
		(1)	(2)	(3)	(4)			(1)	(2)	(3)	(4)			
Between types of mating	1	x_1 6.66	−14.10			Litter size:	$x_1+x_2+x_3+x_4$	15.23	6.99					
		x_2 29.60	− 9.43	4.45	−7.26	Sex:	$x_1+x_2-x_3-x_4$	3.11	−22.43	−49.39	−13.75			
		x_3 2.98	− 4.85	15.27		Skeletal type:	$x_1-x_2+x_3-x_4$	157.79	43.99	− 6.11		66.77	4.66 > 4.26	
		x_4 7.81				Interaction:	$x_1-x_2-x_3+x_4$	12.15						
Between matings of same type	24	x_1 2.02	− 1.13			Litter size:	$x_1+x_2+x_3+x_4$	3.58	− 0.08					
		x_2 1.83	− 1.11	0.76	−0.39	Sex:	$x_1+x_2-x_3-x_4$	2.94	− 2.62	1.28	0.26			
		x_3 2.51	− 0.99	0.90		Skeletal type:	$x_1-x_2+x_3-x_4$	18.06	0.48	1.84		14.34	2.24 > 1.60	
		x_4 1.14				Interaction:	$x_1-x_2-x_3+x_4$	5.42						
Between litters within matings	123	x_1 1.03	− 0.16			Litter size:	$x_1+x_2+x_3+x_4$	3.39	0.13					
		x_2 0.87	− 0.15	0.26	−0.20	Sex:	$x_1+x_2-x_3-x_4$	2.67	0.61	− 0.21	0.41			
		x_3 0.85	− 0.30	0.27		Skeletal type:	$x_1-x_2+x_3-x_4$	6.63	− 0.43	− 0.17		6.40		
		x_4 1.20				Interaction:	$x_1-x_2-x_3+x_4$	3.11						

* Columns (1) contain mean squares, (2) mean products of adjacent variables, (3) mean products of first × third and second × fourth, (4) mean products of first × fourth variables.

The general linear model about the x_a's may be written:

$$x_{aijk} = \mu_{ai} + \delta_{aij} + \beta_{ai}m_{aijk} + \epsilon_{aijk}$$

where x_{aijk} is the transform of n_{aijk}, the number of mice with characteristic a in the kth litter of the jth mating of the ith type of mating, μ_{ai} is a contribution to x_{aijk} due to type of mating, δ_{aij} is a contribution to x_{aijk} due to specific mating such that $\Sigma_j(\delta_{aij}) = 0$, $\beta_{ai}m_{aijk}$ is a contribution to x_{aijk} due to linear regression on serial number of the litter, and ϵ_{aijk} is a random contribution to x_{aijk} such that $\Sigma_k(\epsilon_{aijk}) = 0$. Put in this form, it may readily be seen that our interest lies in the relative magnitudes of the contributions of the μ_{ai}'s to the x_{aijk}'s. There are eight μ_{ai}'s corresponding to 4 kinds of mice $\times$ 2 types of mating. Specifically, one hypothesis about the μ_{ai}'s is to be tested. It is a hypothesis of equal frequencies of the two skeletal types (13/5 and not 13/5) in the two kinds of mating,

$$H: (\mu_{11} - \mu_{21} + \mu_{31} - \mu_{41}) = (\mu_{12} - \mu_{22} + \mu_{32} - \mu_{42}).$$

To test this hypothesis, an analysis of variance and covariance of the five variables $x_1, \ldots, x_4$, m was performed. The details of the analysis are too extensive to be included here but the pattern of the analysis may be seen from the distribution of the degrees of freedom:

Total (89 litters of type 1, 60 litters of type 2)	148
Between matings (13 matings of each type)	25
Between types of mating (2 types)	1
Between matings within types	24
Between litters within matings	123

The analysis was performed by completing the following steps in order:

1. Removal of the effect of serial number m of the litter on the transformed numbers x_1, x_2, x_3, x_4 by adjusting the sums of squares and products by the method of analysis of covariance.

The mean squares and products for the x_a's adjusted for serial number m are shown in table 3, part A, for the three pertinent subdivisions in the analysis. The first two subdivisions—between types of matings and between matings of the same type—are used for detecting differences between types of mating. The last two subdivisions—between matings of the same type and between litters of the same mating—are used for detecting differences between matings. The first two are used in this section; the last two are used in the next section.

2. Rotation of the mean squares of the x_a's to mean squares of certain linear sums or differences of the x_a's. This is done because we are interested, not in the x_a's individually, but in the difference between the types of matings with respect to the relative numbers of type 13/5 mice. Now x_1 and x_3 measure the number of 13/5, while x_2 and x_4 measure the number of not-13/5. Thus a difference in the relative numbers of 13/5 will be shown in terms of $x_1 - x_2 + x_3 - x_4$. A difference in litter size will be shown in terms of $x_1 + x_2 + x_3 + x_4$. Further, a difference in the frequencies of the two sexes will be shown in $x_1 + x_2 - x_3 - x_4$ and an interaction of sex with skeletal type will be shown in $x_1 -$

$x_2 - x_3 + x_4$. The rotation of the mean squares and products of the x_a's to the mean squares and products for the specified sums and differences, shown in table 3, part B, was accomplished by computing, for example,

$$15.23 = 6.66 + 29.60 + 2.98 + 7.81 + 2(-14.10 - 9.43 - 4.85 + 4.45 + 15.27 - 7.26),$$
$$157.79 = 6.66 + 29.60 + 2.98 + 7.81 - 2(-14.10 - 9.43 - 4.85 - 7.26) + 2(4.45 + 15.27),$$
$$-13.75 = 6.66 - 29.60 - 2.98 + 7.81 - 2(-9.43) + 2(-7.26),$$

corresponding to,

$$(x_1+x_2+x_3+x_4)^2 = x_1^2+x_2^2+x_3^2+x_4^2+2(x_1x_2+x_2x_3+x_3x_4+x_1x_3+x_2x_4+x_1x_4),$$
$$(x_1-x_2+x_3-x_4)^2 = x_1^2+x_2^2+x_3^2+x_4^2-2(x_1x_2+x_2x_3+x_3x_4+x_1x_4)+2(x_1x_3+x_2x_4),$$
$$(x_1+x_2+x_3+x_4)(x_1-x_2-x_3+x_4) = x_1^2-x_2^2-x_3^2+x_4^2-2x_2x_3+2x_1x_4.$$

3. Removal of the effect of differences in litter size of the two types of mating on the relative numbers of 13/5 offspring by the method of the analysis of covariance. The three variables remaining at this point are functions of the numbers of males and females (sex), the numbers of 13/5 and not-13/5 (skeleton), and the interaction of sex and skeleton, the functions having been adjusted for variation in serial number of the litter and in litter size.

4. Adjustment of the function which reflects the variation in number of 13/5 mice for covariation with sex and sex-skeleton type interaction. This gives the mean squares shown in table 3, part C.

5. Test the hypothesis of interest. The result is shown in table 3, column F. The test of H gives F = 4.66 which is significant at the 5 percent level and therefore indicates that the two types of mating are different with respect to the incidence of type 13/5 mice after adjustments have been made for serial numbers of litters, sizes of litters, and sexes of mice.

GENETIC SIMILARITY OF HYBRIDS

Two sorts of evidence contribute to the belief that the reciprocal hybrids, while dissimilar in skeletal type, are similar in genetic constitution, or at most that genetic differences are negligible when compared with the large difference between the hybrids.

One line of evidence is contained in the preceding analysis and shows that differences between matings, even though the matings involved animals of 5 sublines of C57 and 2 sublines of C3H, are small relative to the difference between the types of mating. However, there were significant differences between the matings with respect to skeletal type as shown by F = 2.24 in table 3. This may be due to differences between the C3H sublines or between the C57 sublines or to genetic differences between the dams and sires or non-genetic differences between the dams within sublines. The data available do not provide a clear answer to this question since not all possible combinations of 2×5 sublines were represented in the experiment. The question does not seem to be worth pursuing at this point since the difference between the reciprocal hybrids overshadows the differences between the matings of a given type.

The other line of evidence shows that in breeding tests the hybrids are similar and, therefore, that the difference between the hybrids is not a dif-

ference that may be transmitted to the next generation, either by genes or by cytoplasmic factors. To test the breeding behavior of the F_1 mice, two types of F_2 mice were produced by 19 matings among the offspring of C57 ♀ ♀ × C3H ♂ ♂ and by 12 matings among the offspring of C3H ♀ ♀ × C57 ♂ ♂. All matings were between litter mates.

No elaborate statistical analysis of the F_2 data is required because the two groups of F_2 mice are quite similar. They were produced during the same months of the year by females of comparable ages. The average size of litters of descendants of C57 ♀ ♀ was 8.39; of C3H ♀ ♀, 8.44. The sex distribution was very near to equality in both groups. Further, the two groups of mice were similar with respect to distribution of skeletal types. For males, in the F_2 (C57 ♀ ♀ × C3H ♂ ♂) 50 percent were 13/5 and 37 percent were 13/6; in the reciprocal F_2 (C3H ♀ ♀ × C57 ♂ ♂) the percentages were about the same, 53 percent 13/5 and 37 percent 13/6. For females, the corresponding percentages were 42 of 13/5 and 45 of 13/6 in one mating, and 43 of 13/5 and 45 of 13/6 type in the other (table 4). To test the hypothesis of no association of skeletal type in F_2 with origin of parents, we used three skeletal

TABLE 4

Relative frequency of skeletal types in two types of F_2 hybrids from matings of reciprocal F_1 hybrids of C57 Blk and C3H strains of mice. Skeletal type is given as the ratio of thoracic to lumbar vertebrae; 13/$\frac{1}{1}$ and 13/$\frac{1}{2}$ designate mice with asymmetrical sacrums.

Parents		Geno-type		Offspring					Number
Dam	Sire			13/5	13/$\frac{1}{1}$	13/$\frac{1}{2}$	13/6	Other	
				percent					
$F_1 \frac{C57\ ♀}{C3H\ ♂}$ ×	$F_1 \frac{C57\ ♀}{C3H\ ♂}$	*A–*	♂♂	48.9	11.8	2.2	36.7	0.3[a]	313
(19 matings)			♀♀	39.9	10.8	2.3	46.7	0.3[b]	306
(98 litters)			Tot.	44.4	11.3	2.3	41.7	0.3	619
		aa	♂♂	53.5	7.0	1.0	38.4	0.0	99
			♀♀	46.2	10.6	1.9	41.3	0.0	104
			Tot.	49.7	8.9	1.5	39.9	0.0	203
		Tot.	♂♂	50.0	10.7	1.9	37.1	0.2	412
			♀♀	41.5	10.7	2.2	45.4	0.2	410
			Tot.	45.7	10.7	2.1	41.2	0.2	822
$F_1 \frac{C3H\ ♀}{C57\ ♂}$ ×	$F_1 \frac{C3H\ ♀}{C57\ ♂}$	*A–*	♂♂	52.9	5.7	5.2	36.2	0.0	174
(12 matings)			♀♀	42.9	8.3	3.0	45.8	0.0	168
(54 litters)			Tot.	48.0	7.0	4.1	40.9	0.0	342
		aa	♂♂	53.3	3.3	3.3	40.0	0.0	60
			♀♀	44.4	7.4	7.4	40.7	0.0	54
			Tot.	49.1	5.3	5.3	40.3	0.0	114
		Tot.	♂♂	53.0	5.1	4.7	37.2	0.0	234
			♀♀	43.2	8.1	4.1	44.6	0.0	222
			Tot.	48.2	6.6	4.4	40.8	0.0	456

a: 12/6. b: 14/5.

groups (13/5, asymmetrical, 13/6) and found for males that $\chi^2 = 1.26$, and for females that $\chi^2 = 0.18$, both of which are less than the critical value of χ^2 (0.05, 2) = 5.99. While the F_1 hybrids were different in distribution of skeletal types, the difference was not propagated into the F_2 animals. This constitutes evidence that the two groups of F_1 mice were genetically similar.

Since the F_1 mice were heterozygous for the agouti/non-agouti (*A/a*) genes, the offspring in both matings were distributed as approximately ¾ *A–*, and ¼ *aa*. The agouti/non-agouti segregation is not, however, related to the skeletal variation. In each of the two kinds of F_2, the males as well as the females had similar distributions of skeletal types in the two genotypes. Using three skeletal groups as before and a hypothesis of no association of skeletal type with genotype, we found in the F_2 (C57 ♀ ♀ × C3H ♂ ♂) that $\chi^2 = 2.47$ for males and $\chi^2 = 1.26$ for females, and in the F_2 (C3H ♀ ♀ × C57 ♂ ♂) that $\chi^2 = 0.99$ for males and $\chi^2 = 0.67$ for females. These are all less than the critical value χ^2 (0.05, 2) = 5.99.

DISCUSSION

The difference in skeletal type between the reciprocal hybrids may be interpreted in either of two ways. Both interpretations are based upon the assumptions that the difference appears in both sexes, that the difference is not dependent upon circumstances of the environment (litter size, age of mother, and month of birth) which may have a systematic effect on skeletal type, and that the reciprocal F_1 hybrids are genetically similar. There is evidence to support these assumptions. In addition we assume that skeletal type in any mouse is not changed by postnatal events.

One explanation of the hybrid difference is that the cytoplasms of the ova produced by mothers of the two strains are different. The alternative explanation is that the biological environments furnished by mothers of the two strains to the developing embryos are different.

There is no way of discriminating between these alternatives with the present data. The transplantation of ova by the method of FEKETE and LITTLE (1942) or the transplantation of ovaries by the method of RUSSELL and HURST (1945) may help to separate the effects of cytoplasm from the effects of uterine environment. Mice of the same genotype may be gestated in different uterine environments, and mice of different genotypes may be gestated in the same uterine environment by appropriate transplantation of ova or of ovaries. Even though the difference between the hybrids may be found to depend upon the cytoplasm of the ova, it seems clear that the difference is not the sort that can be transmitted to later generations. The reason for this is the similarity of the F_2 generations in this experiment. On the other hand, an ovary-transplantation experiment of RUSSELL (1948) has shown that the maternal uterine environment does influence the distribution of skeletal types, at least in strain 129 especially produced to test this point. It is reasonable to suppose that the uterine environment may, therefore, play an important

role in influencing the skeletal types of the labile F_1 generations in the reciprocal matings of the C57 blk × C3H mice.

SUMMARY

Reciprocal hybrids between the C57 blk and C3H strains of mice were found to differ with respect to the incidence of mice with 5 or with more than 5 lumbar vertebrae. The hybrids tend to resemble the strain of the female parent. The difference between the hybrids is present after corrections are made for serial number of the litter, age of mother, month of birth, litter size, and sex. The difference is not explainable on the basis of subline differences within the pure strains and was not propagated into the second hybrid generation.

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DOMINANCE AS FUNCTION OF WITHIN ORGANISM ENVIRONMENT IN KERNEL-ROW NUMBER IN MAIZE (ZEA MAYS L.) [1]

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DOMINANCE was one of the leading observations of MENDEL and, as such, has become of first importance to genetic teaching and research. It is invoked as an explanation of hybrid vigor. It is utilized and maligned in the inheritance of all forms, particularly man, with ideas of penetrance, expressivity, etc. Most geneticists have formed a mental concept for the term, but few have tested theories covering the vital reactions by which dominance is accomplished. Worthwhile theories which have come from these attempts largely relate to one gene, one primary function reacting system. Genes affecting single chemical synthesis within the organism, *i.e.*, man, Drosophila, or Neurospora, have been shown to act on particular groups through a single chemical bond, each bond generally requiring a specific gene. If the gene mutates to an inactive allele, or is lost, the reaction will not take place. This specificity has led to a " do " concept for the dominant allele and a " do not do " for the recessive allele. In theory the effects of this specificity carry through the whole organism. This concept breaks down somewhat in considering an allelic series where the successive members of the series show graded reactions and seemingly require graded gene products.

But dominance has other possibilities. It may play a prominent part in the enigma of embryonic differentiation wherein the organism is molded into the form characteristic of its species and strain, from an apparently undifferentiated zygote. The significance of this theory becomes clear in investigations of the genetics of kernel-row number in maize. The discussion to follow deals with this problem.

Investigations in the nature of inheritance of kernel-row number in maize have generally indicated that kernel-row number is a polygenic characteristic (EMERSON and EAST 1913; LINDSTROM 1931). (The term polygenic as used here is synonymous with the lengthier term multiple-gene-determined.) This information was based on genetic analysis of lines with eight or more kernel-rows.

TAVCAR (1935) reported a true breeding four-rowed corn variety out of a red flint type. In crosses of four- by eight-rowed types he obtained, for the

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most part, hybrids which had eight rows on the base of the ear and four rows at the tip. Pictures show that in his material the shift from eight to four rows is clear-cut with two bilaterally opposed rows of alicoles dropping out to leave a four-rowed tip. His segregating material led him to postulate a two-gene hypothesis for row number inheritance between four and eight rows.

A different four-rowed line from the one used by TAVCAR (1935) was isolated from Golden Bantam and inbred by the late DR. E. W. LINDSTROM. This line was so different in appearance and origin from TAVCAR's line that a study of its genetic make-up with respect to row number was undertaken. Crosses of this four-rowed line with eight-, twelve-, and fourteen-rowed types are reported together with linkage tests between row number genes and several marker genes.

To improve the observations further, a greenhouse experiment was conducted in which the attempt was made to induce all the differentiated ear meristems of the plant to develop so that the row numbers of the ears at the various nodes could be determined. This material is pertinent to the problem of dominance and ontogeny.

MATERIALS AND METHODS

These studies may be divided into two phases. The first was conducted in the field in 1946 and the second in the greenhouse in 1947.

Field: The inbred lines of corn used with their gene designations were:

White Flint ..... WF 8-*y-p-Su*
Yellow Dent ..... Idt 14-*Y-P*^{r}*-Su*
Yellow Dent ..... Osf 12-*Y-P*^{r}*-Su*
Four-row Sweet 4-*Y-p-su*

Arabic numbers indicate the modal number of kernel-rows characteristic of the line. *Y* is the sixth chromosome gene for yellow (*Y*) or white (*y*) endosperm color. *P*^{r} is a member of the multiple allelic series of cob and pericarp color-pattern genes on the first chromosome, *P*^{r} being red cob, and *p*, white cob. *Su* is the fourth chromosome gene for endosperm texture; starchy (*Su*) versus sugary (*su*).

These lines are material of the GENETICS SECTION, IOWA AGRICULTURAL EXPERIMENT STATION, which were inbred and maintained by the late DR. E. W. LINDSTROM and his staff. WF is a long-time inbred out of White Flint. Idt is a field dent line out of the variety, Iodent. Osf came from Osterland's Reid. The 4-*Y-p-su* line is a four-rowed variant isolated from Golden Bantam and inbred.

The eight-, twelve-, and fourteen-rowed lines were crossed with 4-*Y-p-su* and the F_1's either selfed or backcrossed to the four-rowed parent. All references to backcrosses are to this type unless otherwise specified.

The F_2 and backcross generations of these three crosses along with the parents were grown in the field during the summer of 1946. The mature, open pollinated top ears were harvested in the fall and evaluation was made of kernel-row number and genotype with respect to the marker genes. It was

impossible to determine heterozygous *Yy* and *Susu* plants by observing segregation in the open-pollinated ears.

Kernel-row number classification was made in classes numbered continuously from four through sixteen, the odd numbers indicating intermediates. Row number data taken in the field were analyzed by means of a t-test (SNEDECOR 1946) on the difference between the mean row number associated with each qualitative gene pair.

Greenhouse: Two different lines were used in greenhouse studies along with 4-*Y-p-su:*

Hickory King ..... HK 8-*y-p-Su*
Yellow Flint ..... 8-*Y-P*^{r}*-Su*

HK is an inbred out of the flint variety, Hickory King. The 8-*Y-P*^{r}*-Su* line is a " synthetic " inbred, maintained in part for its unusual combination of low row number and red cob.

Five groups were planted in the greenhouse: (1) 4-*Y-p-su*, (2) Hickory King, (3) (4-*Y-p-su* × HK) F_1, (4) (4-*Y-p-su* × 8-*Y-P*^{r}*-Su*) F_2, and (5) the backcross (4-*Y-p-su* × 8-*Y-P*^{r}*-Su*) F_1 × 4-*Y-p-su*.

The greenhouse experiment was planted on January 5, 1947, using 2½-inch pots, later transplanting to six-inch pots in a " randomized block " design with four replications.

A photoperiod of daily decreasing length was provided for the first twenty days and thereafter, with the seasonal increasing photoperiod.

Date of anthesis (numerical day of the year) was recorded for each plant, daily at 1700 hours, and at the same time the tassel was removed. By limiting the amount of pollination, the lower ears were induced to develop.

DOMINANCE

The F_1 generation and both parents from the cross HK 8-*y-p-Su* × 4-*Y-p-su* were grown in the greenhouse. The data from this material consisted, in part, of the row number of each potential ear on the plant. Most plants were induced to differentiate from four to six ears.

Figure 1 shows the mean kernel-row number for each ear position, 1 being closest to the ground level, in the parents and the F_1 (data in table 1).

General observations made under field conditions where but one or two ears developed, have led to the conclusion that the eight-rowed condition is dominant over the four. This observation is based on row number determinations of the top ear, the one which usually develops. Figure 1 shows that under conditions of the greenhouse experiment, dominance depends on ear position within the plant. The top ears of F_1's, developing under the most favorable physiological conditions, are primarily eight-rowed. The lower ears, which initiate row number later (HERSHEY 1934) under different conditions, are usually fours.

The data of table 1 presented in figure 1 show the within plant variation in F_1 is much greater than within plant variation in either of the parents. The inbred parental line used as the low row number parent varied from

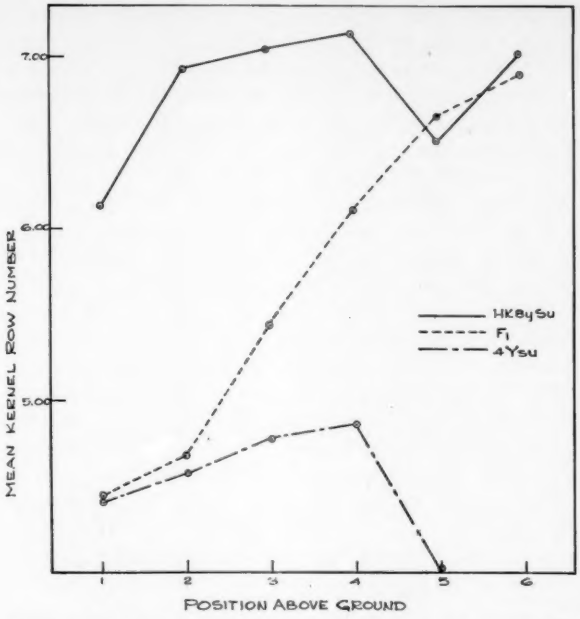

FIGURE 1.—Mean kernel row number at various positions on the plant. HK 8-*y*-*Su* × 4-*Y*-*su*, parents and F_1.

4.00–4.40 to 4.86, the 4.00 average being out of the trend shown by the whole data. The trend showed a distinct effect of ear position above ground on the ear row number, the higher the position, the larger the average row number. The variation was from possibly 0.5 to 0.9 of a row. The inbred line used as high parent had row numbers which also varied with height of ear from the ground, 6.14 at the lowest position to 7.12 at the highest. The row numbers increased with distance of the ears from the ground. Judged from the trend,

TABLE 1

Mean kernel-row number of ears at various positions on the plant.
(See fig. 1)

		Position above ground level					
		1	2	3	4	5	6
HK 8-*y*-*p*-*Su*	n	101	101	95	66	20	2
	$\bar{x}$	6.14	6.95	7.03	7.12	6.50	7.00
4-*Y*-*p*-*su*	n	119	118	113	49	3	....
	$\bar{x}$	4.40	4.58	4.78	4.86	4.00	....
(HK 8-*y*-*p*-*Su*	n	116	115	113	88	44	9
× 4-*Y*-*p*-*su*)F_1	$\bar{x}$	4.43	4.68	5.45	6.10	6.64	6.89

the average difference from the lowest ear to the highest would be not more than half a row number. The F_1 row numbers showed much greater variations. The ears varied from the row number of the lowest parent to that for the highest parent. The F_1's expressed the possible row numbers of *both parents*. The change in row number of the F_1 was essentially linear with the position of ear above ground. The variation observed was more than four times that of either inbred parent line. This effect is the expression of the heterozygosity of the row number genes which we call dominance. A greater range of potential response is open to the hybrid than to homozygous or approximately homozygous plants. The various ears in the above ground positions are differentiated at different times, in basipetal succession according to HERSHEY (1934). The various physiological balances (the environment in which genes produce their effects) change as the plant grows; the ratio of soluble and condensed carbohydrates to soluble and condensed amino acids is a case-in-point. Since parents and F_1 were grown side-by-side under presumably the same external environmental conditions, the response of the F_1 is a function of the genetic environment. The parents were essentially homozygous and the F_1 is heterozygous, at least for the genes determining four and eight kernel-rows. Heterozygotes are more variable than homozygotes on a within plant basis. If a greater response potential is a usual property of hybrids, it could account for hybrid vigor. By being physiologically more adaptable, the hybrid would flourish under conditions that would only sustain homozygotes.

This basipetal decrease in kernel-row number has been observed by the author in other, higher row number material to such an extent that it appears to be the general rule. CUTLER and CUTLER (1948) have called attention to the fact that a decrease in kernel-row number toward the base of the plant can be observed in a wide range of row number types.

The apparent dominance of the eight-rowed condition in the (8×4) F_1 is governed by the physiology of the plant in a relatively advanced stage of growth (HERSHEY 1934). This may mean that, even though row number is genetically determined by one or two major genes, progressive dominance changes throughout the plant have considerable effect. This case is particularly favorable for showing how dominance may act.

These data have importance to the developmental mechanics of the unfolding organism. The F_1 individuals show that the different parts of the plant are not alike. The chemical systems within the cells which form these different parts give precedence to the effects of one genome in one place, the other genome in another and something in between in other locations. This fact is significant to any consideration of ontogeny.

The corn row number is particularly favorable material to bring out this effect, but there is reason to think it may be quite general. The gradients do not have to be linear along the body length. ALLEN (1914) pointed out that spotting of coat color in mammals and birds shows high centers of activity irregularly distributed over the body. These centers suggest the dominance of

TABLE 2

Percent frequency of four- and eight-rowed plants in segregating generations of two hybrids.

Cross	Generation	4-row	8-row	Number of plants	χ^2 (1:3) (9:7)
WF 8-*y-p-Su* × 4-*Y-p-su* (field)	BC**	68.1	31.9	229	5.76*
	F_2	49.6	51.4	558	4.52*
8-*Y-P*^{r}*-Su* × 4-*Y-p-su* (greenhouse)	BC	76.1	23.9	117	0.07
	F_2	42.7	57.3	178	0.09

* Significant, 5% level.
** Backcrosses are all to 4-*Y-p-su* line.

one allele in one area, that of the other allele in another area. The reacting system gains clarity in the corn case by the ordering of the dominance effects of the two genomes, but this ordering may be accidental.

Gene basis for the row numbers

The number of genes involved in the row number inheritance come into consideration when the segregates of F_2 and backcrosses are considered. The case studied to date is the limiting one of the kernel row number of the top ear.

Number of genes

Several investigators have postulated the polygenic nature of kernel-row number inheritance (EMERSON and EAST 1913; LINDSTROM 1931). With the four-rowed material used in this investigation, however, there was a suspicion that a single major gene might determine the threshold between four and eight kernel-rows.

Table 2 shows the frequency of four- and eight-rowed plants in various segregating generations. The grouping of the intermediates between four and eight was made on the basis of the row number distributions of field-grown parent populations (fig. 5). Here it was noted that the 4-*Y-p-su* parent gave a relatively high percentage of intermediates (row number class 5 and 6) and the eight-rowed parent gave relatively few (table 3). The percentage of intermediates in backcross and F_2 generations is essentially the same as in the

TABLE 3

Percent frequency of row number classes in four- and eight-rowed parents. (See also fig. 5)

Parent	Row number class						
	4	5	6	7	8	9	10
4-*Y-p-su*	64.1	26.9	9.0	0.0	0.0	0.0	0.0
WF 8-*y-p-Su*	2.4	8.5	3.7	0.0	82.9	2.4	0.0

four-rowed parent. It seems reasonable, therefore, to group these intermediates with the fours, and the seven class, with the eights.

With this grouping (table 2), a remarkably good fit to a 9:7 F_2 (1:3 BC) ratio is observed in the greenhouse material (two genes, both dominant with complementary action). A rather poor fit to this ratio is evident in the field study. However, these two crosses involve different eight-rowed parents and the segregation populations were grown under widely different conditions (greenhouse and field). This suggests alternative explanations for the fact that the data do not agree. The possibility exists that the modifier backgrounds differ sufficiently to cause the dissimilar frequencies of the two ear types among the segregates. On the other hand, these eight-rowed parents may carry different genes for the eight-rowed condition. It is evident, at least, that the number of genes involved is small.

Linkage relations of row number genes with certain qualitative genes

The F_2 and backcross generations of three different inbred crosses to the 4-*Y*-*p*-*su* line were grown in the field in the summer of 1946, along with the four parents involved. These crosses were:

WF 8-*y*-*p*-*Su* × 4-*Y*-*p*-*su*
Idt 14-*Y*-*P*^{r}-*Su* × 4-*Y*-*p*-*su*
Osf 12-*Y*-*P*^{r}-*Su* × 4-*Y*-*p*-*su*

Interest centered about testing for linkage of row number genes with the *P*, *Y*, and *Su* linkage groups. LINDSTROM (1931) has reported strong indications of linkage with the *P*-locus when eight-, ten-, twelve-, and fourteen-rowed lines are intercrossed.

The data on linkage are presented in table 4. In no instance were the monogenic ratios out of the range of expectation. Reference has been made under "Material and Methods" to the system of estimating row number and the grouping used. Error from this source was considered less using the t-test than with a $2 \times n$ contingency table of χ^2. However, there was quite good agreement between the probability values for χ^2 and the t-probabilities shown in table 4.

Backcross and F_2 data were obtained from three different crosses as shown in the table. The mean kernel-row number associated with each allele of the segregating genes is given and the standard error of the differences in these mean kernel-row numbers is calculated. The significance of these differences is indicated as well as the "direction" of association. A minus "direction" indicates greater non-parental association between row number and the qualitative gene and a plus "direction" reflects greater parental association. For instance, in the cross WF 8-*y*-*p*-*Su* × 4-*Y*-*p*-*su* high row number and *Su* are associated in the parents, while in the F_2 high row number is associated with *su* which is a non-parental combination.

In the instances where the non-parental class exceeds the parental (WF 8-*y*-*p*-*Su* × 4-*Y*-*p*-*su*) and the difference is significant, no well founded explanation is available for the reversal of linkage, consequently, any elaboration

TABLE 4

t-test for detection of linkage between kernel-row number genes and Y, P, and Su linkage groups.

Cross	Generation and number of plants	Genotype	Mean row number	Difference between means	P t-test	Direction[1]
WF 8-*y-p-Su* × 4-*Y-p-su*	558-F_2	*Y*	6.66	0.42 ± .212	.05	−*
		y	6.24			
		Su	5.46	1.41 ± .202	.01	−**
		su	6.87			
	225-BC	*Y*	5.88	0.47 ± .226	.05	−*
		y	5.41			
		Su	5.61	.04		−
		su	5.65			
Idt 14-*Y-P*^{r}-*Su* × 4-*Y-p-su*	180-F_2	*P*	10.54	0.49 ± .290	.10	+
		p	10.05			
		Su	10.64	0.43 ± .279	.15	+
		su	10.21			
	212-BC	*P*	7.55	0.10 ± .237	.50	−
		p	7.65			
		Su	7.61	.04		+
		su	7.57			
Osf 12-*Y-P*^{r}-*Su* × 4-*Y-p-su*	213-F_2	*P*	9.11	0.32 ± .241	.20	−
		p	8.79			
		Su	9.17	0.31 ± .310	.40	+
		su	8.86			
	210-BC	*P*	7.19	0.57 ± .228	.02	+*
		p	6.62			
		Su	6.89	.04		−
		su	6.93			

[1] Direction indicates the parental minus the non-parental class.
* Significant, 5% level.
** Significant, 1% level.

of this point would be purely speculative. It is interesting that this same reversal of linkage has been reported before in *SuSu* × *susu* crosses (LINDSTROM 1931).

The data show a slight indication of linkage between row number genes and the *P* locus; however, the relation is not consistent and no conclusions may be drawn. None of the other genes gives any indication of being linked with row number genes.

Row number frequency distributions of segregating generations

Figures 2, 3, and 4 show the percentage frequency distributions of row number from the backcross and F_2 generations of the three crosses discussed under linkage. These distributions and even their means are apt to be misleading from a genetic point of view, since classification of a presumably

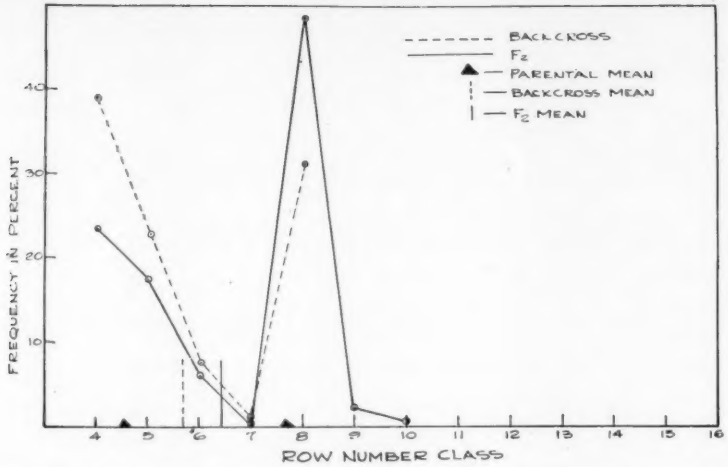

FIGURE 2.—Row number distribution of F_2 and backcross generations. WF 8-*y-p-Su* × 4-*Y-p-su*.

polygenic characteristic has been made in discrete classes and these classes, themselves, are further prescribed by certain structural possibilities in the plant. For instance, the five-row class does not indicate complete disruption of ear morphology to lay down five kernel-rows, although odd numbers of

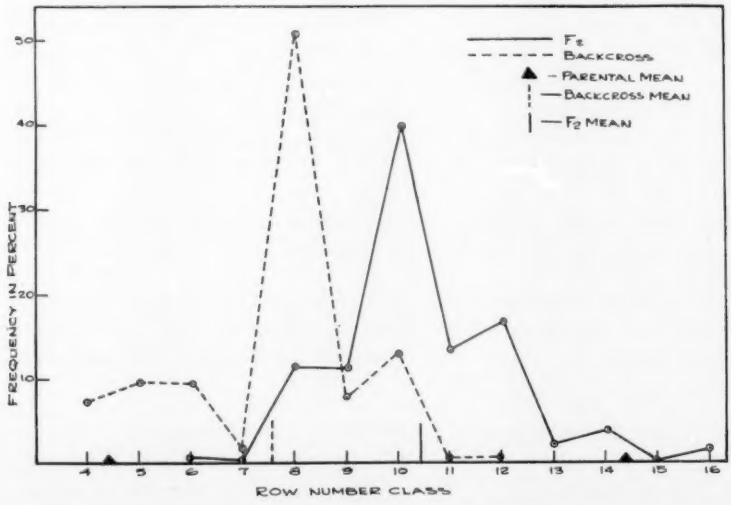

FIGURE 3.—Row number distribution of F_2 and backcross generations. Idt 14-*Y-P'-Su* × 4-*Y-p-su*.

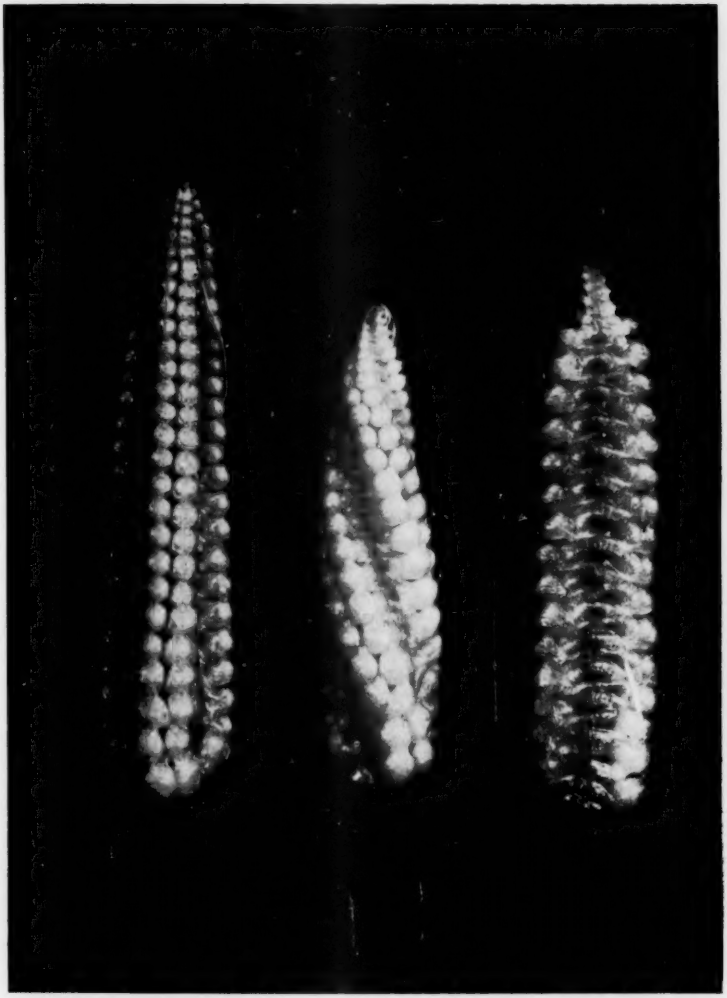

PLATE I

Young corn ears showing the typical eight- (left), six- (center), and four-rowed (right) conditions. Notice the characteristic false spiral of the six-rowed ear.

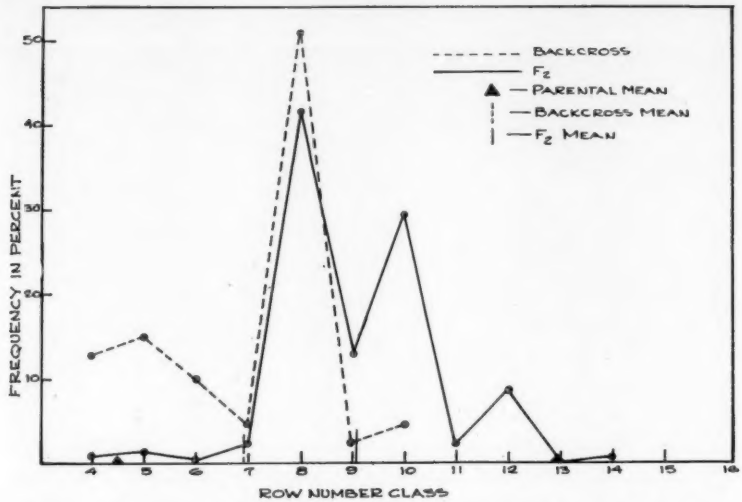

FIGURE 4.—Row number distribution of F_2 and backcross generations. Osf 12-*Y-P'-Su* × 4-*Y-p-su*.

rows have been observed and accounted for, structurally, in certain South American varieties (CUTLER 1946). The odd numbers here indicate intermediates, usually classifiable as such. Furthermore, among the even numbers, ten-, and especially six-rowed ears show a certain amount of distortion in the form of what some investigators have called a false spiral (Plate I) to distinguish it from the true spiral caused by fasciation of the spike. The six-rowed ear has not been found in true breeding strains and neither does it show up as a large class of intermediates in crosses of 4×8. Sixes occur just as often in the four-rowed parent as they do in the F_1 or F_2 between four and eight.

The basic pattern of four, eight, and twelve rows is clearly apparent. Classification should probably be made in these groups if it were not for the bias that would enter from the placing of the intermediates. Leaving out the intermediates is another possibility but again, if the intermediates were characteristic of one group and not the other, bias would enter. A clue to this may be seen in row number distributions of the four- and eight-rowed parents (fig. 5). Here, apparently, the intermediates occur most frequently with the four-rowed condition. This would indicate that the intermediates in F_2 should be divided between the four and the eight classes on the basis of their percentage of the parents.

DISCUSSION

The fact that embryological development may be regarded as a series of progressively changing steps, in itself suggests that the genes contained within

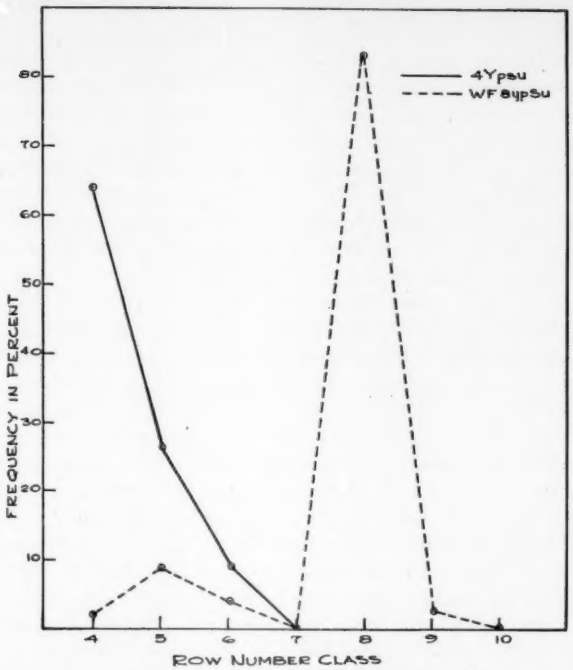

FIGURE 5.—Row number distribution in four- and eight-rowed parents. WF 8-*y-p-Su* and 4-*Y-p-su*.

the cells making these steps are a part of a progressively changing chemical environment. Genes require substrates from which to build future products. With these substrates under progressive change, it is likely that the catalyzing of a process in one stage by one allele may be increased, while at another stage it may be limited. Changes in the developmental environment may even result in complete loss of function by one allele, thus giving the other allele the control of the master reaction. Under these circumstances, allele action within the framework of development may be expected to vary. This variation would allow certain genes to be the masters of one embryological stage and the servants of another. By this functional arrangement, the flexibility necessary for form and function control may be introduced into the system. The organism may be a mosaic of such systems. When this is the case it becomes difficult to even visualize the system. On the other hand, the system may be ordered in some linear manner, thus making the successive gene expressions clear. The kernel-row number development in corn herein described is such an ordered case.

The ordering of the system becomes evident in either of the pure homozygous inbred lines, but the range of the effects is so much reduced that it

could be overlooked. It is only in the heterozygote that the full range of effect becomes evident. In the F_1 both parental haploid sets come into prominence at different developmental stages. Dominance is expressed by the low row number allele when the ear is forming in the low position on the plant. High kernel-row number is expressed when the forming ear is at the top of the plant. A gradient of internal environment is established between these limits such that the genes for the kernel ear row numbers show a progressive shift in their developmental effects. Flexibility in gene developmental control is introduced. The interactions of the genes in action, gene-controlled substrates and environment are such as to allow diversity in the development of organ systems, etc., throughout other parts of the body.

Gradients in development were postulated early, but the complexity of the systems involved have not favored further analysis. The so-called axial gradient is such a case. But it does not appear that the gradients necessarily need be linear. They could be mosaic in form and could change with advancing development. Coat color patterns in mammals and birds as mentioned earlier are illustrations of this possibility.

The hypothesis proposed here, that alleles may have slightly different functions and that the relative dominance of an allele is dependent upon the developmental stage of the organism, can be invoked as explanation for the complementary action of mutant alleles. WHITING (1951) has given data on two loci of three alleles each in Habrobracon and one locus of four alleles in Mormoniella where the mutant alleles in combination produce a wild-type phenotype. He attributes this action to complementary dominance of the two mutant alleles but has not given evidence as to the developmental stage at which each allele is expressed. If this was possible, as it has been shown in the corn kernel-row number data, then each allele probably would impinge at a different stage in the development, appearing as dominant at that particular time.

Still another explanation may be fitting for the kernel-row number data and the cases of complementary action of alleles in Habrobracon. GOLDSCHMIDT (1927) postulated a general theory of dominance which states that the expression of an allele in heterozygous condition depends upon the rate of a reaction in development and upon the point of cessation of that reaction in the heterozygote. If this theory were applied to the corn data where ears are both four-rowed and eight-rowed on the same heterozygous plant, we would have to say that in developing the four-rowed ear, developmental rate was slower or development ceased sooner or both factors were brought into play in one part of the plant, while in another part of the same plant another set of conditions prevailed. This explanation is not so inconceivable as it is irrelevant since it would not necessarily predict the appearance of both alleles in a heterozygote as would the hypothesis stated here.

The kernel-row numbers in the parental inbreds as contrasted with those in the F_1's furnish an impressive demonstration of how the two genomes in a heterozygote can interact and be given expression. Elasticity in phenotypic expression is greater in the heterozygote than it is in the more homozygous inbreds. Seemingly the F_1's should be capable of giving the best phenotype

for a variety of environments. This is certainly one of the prime considerations in hybrid vigor in corn. It seems that the increased variation shown in the hybrids must be due to either heterozygosity *per se* or to the fact that the hybrid contains more different alleles than either parent. Whichever explanation is appropriate, our conclusion must postulate that the alleles at the average heterozygous locus have different functions. We can say that the 8-row genes are dominant at the upper positions on the plant and the 4-row genes are dominant in the lower positions, but this is merely a restatement of the hypothesis of different functions. If the 8-row genes are dominant in the upper positions, then their function is different in the lower positions.

Even though we cannot determine whether the increased variation is due to within locus or between locus causes, one thing seems relatively certain from these data: the greater number of different alleles in the hybrid is the cause of the greater within plant variation in kernel row number. Greater within plant variation presumably opens a broader developmental field to the hybrid and thereby the basis for greater vigor.

The kernel-row number data have a bearing on recessive-dominance concepts. The data show that the within plant environments may give phenotypic expression to one genome at one time or to the other genome at another.

Row number genes for corn strains with customarily eight or more rows on the top ear under field conditions, appear to be fairly numerous according to the works of EMERSON and LINDSTROM. To test for these genes, and, if possible, to locate them within the chromosomes, LINDSTROM (1931) tested the first, fourth, sixth, and tenth chromosomes in maize. Using the qualitative genes *p, su, y,* and *r* in a large number of crosses involving row number differences (eight rows being the lowest), a correlation between the *P* locus and row number was found which was interpreted to indicate that one of the major row number genes was located near *p* on the first chromosome. Negative or inconsistent results were obtained with the other chromosomes.

In the crosses of the 4×8 kernel-row ear types presented here two loci appear to be needed to differentiate the four and eight rowed types. Backcross and F_2 data fail to show linkage of row number with loci for *P, Y,* and *Su*. These observations agree with TAVCAR's (1935) data on the *P* and *Y* loci.

SUMMARY

The data on kernel-row number, as determined under conditions favorable to the development of four to seven ears on a plant, show the relatively homozygous inbreds of four and of eight rows are stable with 0.5 of a row number. The F_1's, on the other hand, show dominance of the eight-rowed at the top of the plant, and of the four-rowed type at the lowest position. Between these extremes, row number increases linearly with the successive nodes up the plant. A comparable trend, but of much reduced range, is noted for the inbred parents.

These facts show that dominance within the plant follows a within plant gradient. Gene action at successive stages of development is dependent on the condition of the substrate on which it works. The inbred's flexibility in meet-

ing these environmental changes is much less than that of the heterozygotes. F_1 adaptability should allow for vigorous activity over a much larger range of conditions. The heterozygous genomes of the F_1 each express their potentialities at different phases of growth.

In crosses of an eight-rowed line by a four-rowed inbred, kernel-row number gave some indications of being under the control of two complementary genes segregating in a 9:7 ratio in F_2 and 1:3 in the backcross.

Inconclusive data resulted in the linkage study between row number genes and the *P*, *Y*, and *Su* linkage groups. A significantly higher proportion of non-parental types was obtained from some crosses.

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ALLELIC AND NONALLELIC GENES CONTROLLING HOST SPECIFICITY IN A BACTERIOPHAGE *

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BACTERIA infected with any two independently arising *r* (plaque type) mutants of the bacterial virus T2H have been found, as a rule, to yield wild-type and double-mutant individuals among the viral progeny (HERSHEY and ROTMAN 1948). This result has been interpreted in terms of recombination among nonallelic genetic factors, and it is permissible to speak of the test in which bacteria are infected with two different viral mutants as a genetic cross.

Preliminary crosses (HERSHEY and ROTMAN 1948, and unpublished) between pairs of independently arising host-range mutants of the same virus, however, have failed to yield recombinants, even when the two mutants were clearly different. LURIA (personal communication) has had the same experience with host-range mutants of the related phage T2L.

If the results with the two kinds of mutant are to be given a consistent interpretation, it has to be assumed that different host-range mutations, unlike the *r* mutations, tend to occur at a single genetic locus. This assumption is not implausible, inasmuch as the host-range character can be readily combined with the *r* character in the appropriate genetic crosses (HERSHEY and ROTMAN 1949).

Nevertheless, we felt that the genetic interpretation could be greatly strengthened by establishing one authentic example of multiple allelism in the virus. This we have done, by three independent means. The demonstration is all the more convincing because one mutant was found that proved to be carrying a mutation in a second locus controlling host specificity.

METHODS

The technique of crossing viral mutants has been modified slightly since last described (HERSHEY and ROTMAN 1949). The bacteria are now grown in nutrient broth, suspended in buffered saline, admixed with virus, and diluted in broth after allowing five minutes for adsorption to occur. Adsorption in the absence of nutrient is physiologically equivalent to simultaneous adsorption of the added virus particles (BENZER *et al.* 1950), and the effects we observe are understandable on the assumption that this causes a more

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nearly equal intracellular growth of the two parental types of virus in individual bacteria. The yields of recombinants obtained by this method are about 1.3 times larger than those previously reported. The linkage relations remain unchanged.

Serial passage of viral stocks for the analysis of patterns of back-mutation is carried out by growing bacteria in 10 ml of nutrient broth to a titer of about 10^7 per ml, and seeding the culture with about 0.03 ml of the lysate of the preceding day. The cultures are incubated overnight at 37°C, sampled for plating, and then heated for 10 minutes at 60°C before transplantation. Since the rate of population change varies with the conditions of propagation, only parallel series are used for comparing the behavior of different viral lines.

All the viral mutants described below are derived from a line called T2H, otherwise referred to as the wild type. The term " sensitive bacteria " means *Escherichia coli* strain S (for seeding agar plates) or strain H (for all other purposes). " Indicator bacteria " means strain No. 2 B/2H,2K, which is resistant to wild-type T2H. " Mixed indicator " means a mixture of sensitive and indicator bacteria.

Plaques arising from stocks of wild type on single indicator plates contain host-range (*h*) mutants only (LURIA 1945). Pure lines of *h* mutants are isolated by sampling from these plaques with an inoculating needle, replating the diluted sample on sensitive bacteria, and resampling from one of the secondary plaques.

Different *h* mutants may be classified in terms of the ratio of the plaque count on indicator plates to the plaque count on plates seeded with sensitive bacteria. This ratio, called the efficiency of plating (e.o.p.), is a heritable characteristic of the virus (HERSHEY 1946) and varies between 0.01 or less and unity for different mutants.

Plaques arising from mutant stocks on single indicator plates, and plaques arising from either wild or mutant stocks on mixed indicator plates, contain principally phage of the parental type, but sometimes also an appreciable proportion of mutants that originated within the plaque. The e.o.p. of a clone sampled from indicator plates is therefore measured only after replating on sensitive bacteria and sampling from one of the secondary plaques. This procedure gives consistent measures of the e.o.p. of different clones of the same line sampled from indicator plates.

DESCRIPTION OF MUTANTS

Mutants with e.o.p. measuring 0.3 or less form turbid plaques (phenotype h^t) on indicator plates. Mutants with e.o.p. near unity form clear plaques (phenotype h^c) on indicator plates. Mutants with intermediate e.o.p. form plaques of intermediate type. Also on mixed indicator plates, the plaques are more turbid the lower the e.o.p., with the difference that the number of plaques is the same as it is on sensitive bacteria. A differential count of mixtures can therefore be made on mixed indicator plates if the component viral types have sufficiently dissimilar e.o.p. On sensitive bacteria, all the mutants form plaques indistinguishable from those of the wild type.

An additional point of difference among the *h* mutants is revealed by heating stocks diluted in phosphate buffer of pH 7.0 for 10 minutes at 64°C, and measuring the proportion surviving. (Identical results are obtained when undiluted broth stocks are heated.) This test shows that the *h* mutants are less resistant to heat than the wild type, and that the h^c mutants are less resistant than the h^t. Five independently arising mutants of the phenotype h^c proved to be equally heat-sensitive. Several *r* mutants, however, were as resistant as the wild type.

When large amounts of wild-type stocks are plated out on indicator bacteria, the numbers of plaques of h^t and h^c mutants are about equal, in the proportion of about 10^{-6} to the titer of the stock. The proportion of h^c mutants in the stock is therefore about 10^{-6}, and the proportion of h^t mutants is at most 10^{-4}. The proportion of h^c mutants in initially pure clones of h^t is less than 10^{-5}. These circumstances make it unlikely that an h^c mutant isolated from a stock of wild type arose by a second mutation from the h^t virus present, whereas an h^c mutant coming from a stock of h^t clearly did so. Both classes of mutant are used in the following experiments. The origins and properties of several lines are summarized in table 1.

TABLE 1

Origin and properties of several h mutants.

Line	Origin	e.o.p.	Percent survival at 64°C, 10 min.
$h^c1 \ldots h^c5$	Wild type	1.0	1.4–5.3
h^c6	h^t1	1.0	1.1–2.0
h^c7	h^t5	1.0	3.0–5.7
h^t1	Wild type	0.1	13
h^t2	Wild type	0.3	Not tested
h^t5	Wild type	0.01	24–30
$h^t1\ h^t5$	cross	0.5	9.5–25
Wild type	....	$< 10^{-5}$	40–48

$h^c1 \ldots h^c5$ are five independently arising mutants of identical phenotype.

The column headed e.o.p. gives the ratio of the titer on indicator bacteria to the titer on sensitive bacteria.

The data for survival at 64° for 10 minutes show the range of results obtained with two or more different stocks of each line, including stocks propagated in glucose-ammonia medium as well as stocks propagated in nutrient broth.

ADSORPTION OF *h* MUTANTS

LURIA (1945) showed that a host range mutant of T2 was adsorbed at measurable rate to its indicator strain of bacteria, whereas the wild type was not, and suggested that this is the primary difference between the two viruses. He also found that the rate of adsorption of the mutant to indicator bacteria was considerably slower than to sensitive bacteria, and wished to explain the low and variable efficiency of plating of the mutant on this basis.

We have measured the adsorption of several *h* mutants to bacteria with the expectation that the rates would parallel the e.o.p., and that either could be taken as an expression of different degrees of adaptation of virus to host.

In a general way this expectation proves correct, but the effects of mutation do not appear to be entirely explainable on this simple basis.

For the measurement of slow rates of adsorption, we have used the following method. Phage and bacteria are mixed, held for a measured time at 37°C, and the unadsorbed phage is inactivated by the addition of antiphage immune serum. The suspension of cells is then titrated before the end of the latent period of phage growth to determine the number of cells yielding phage. Provided the proportion of cells infected is kept small, their number gives an estimate of the number of phage particles adsorbed. The results given by this method agree with those obtained in the usual way, by titrating unadsorbed phage after centrifugation, when the rates can be measured by both methods: namely, in the adsorption of wild type or *h* mutants to sensitive bacteria, and in the adsorption of h^c to indicator bacteria.

As far as could be determined, a solution containing 0.15 M NaCl and 10^{-4} M $CaCl_2$ supplies all the requirements for adsorption to bacteria of T2H and its host range mutants. The calcium is needed only for the adsorption to indicator bacteria; the adsorption of *h* mutants to sensitive bacteria is practically unaffected by calcium. The rate of adsorption is not affected by added nutrient broth (or L-tryptophan) provided the broth suspension of bacteria is kept at low temperature until the moment of adding phage. Incubation at 37°C of washed bacteria with broth (or bacteriological peptone, or L-tryptophan) for a few minutes produces a powerful soluble inhibitor of adsorption that may be indol, since indol also has an immediate inhibiting effect. The sensitivity to indol is a characteristic of T2H not shared by all its relatives. The *h* mutants are somewhat less sensitive than wild type to the inhibitor derived from peptone when tested by adsorption to sensitive bacteria. They are equally sensitive among themselves when tested by adsorption to either sensitive or indicator bacteria. For this reason, the inhibitor cannot be a primary factor in determining the e.o.p. of the *h* mutants.

Table 2 shows the rates of adsorption of several phages to bacterial suspensions prepared from cultures of various kinds. In each case the bacteria were washed and resuspended in a saline phosphate buffer containing 10^{-3} M

TABLE 2

Rates of adsorption of h mutants to bacteria.

Bacteria	Phage				
	Wild	h^c1	h^c2	h^t1	h^t5
Sensitive					
2 hour broth	3×10^{-9}	....	3×10^{-9}	3×10^{-9}	3×10^{-9}
Indicator					
2 hour broth	$< 2 \times 10^{-14}$	5×10^{-10}	6×10^{-10}	6×10^{-14}	1×10^{-12}
day old broth	$< 2 \times 10^{-14}$	....	5×10^{-10}	7×10^{-13}	1×10^{-12}
synthetic medium	1×10^{-13}	....	1×10^{-9}	6×10^{-11}	1×10^{-11}

The rates given are per bacterium per ml per minute at 37°C.

$MgSO_4$, 10^{-4} M $CaCl_2$, and 10 mg of gelatin per liter, in which the phages are stable over long periods. Most of these rates have been verified several times using various concentrations of bacteria and phage. That the slow rates are not artifacts due to the rapid adsorption of phage to a small fraction of sensitive bacteria in the population is shown both by the measurements with wild type, and by the fact that the number of bacteria infected is proportional to the amount of phage added over the ten-fold range subject to test. In particular, measurements of bacterial survival show that at least 80 percent or so of the cells in a culture grown in a glucose-ammonia medium adsorb the mutants h^t1 and h^t5 at the rates shown in the table.

The main features of the data in table 2 are the following:

All the *h* mutants, as well as the wild type, are adsorbed at equal rates to sensitive bacteria.

All the *h* mutants, but not the wild type, are adsorbed at measurable rates to indicator bacteria, and the mutants h^c are adsorbed much faster than the mutants h^t.

The mutant h^t5 is adsorbed to indicator bacteria from broth cultures at a faster rate than the mutant h^t1. This is contrary to the expectation based on measurements of e.o.p.

The relative rates of adsorption of the different *h* mutants to indicator bacteria varies with the age of the culture from which the bacterial suspension is prepared. The rate increases with age for the mutant h^t1, and remains constant for the mutants h^c2 and h^t5. Both types of behavior are contrary to general experience; in fact, the rate of adsorption of these same mutants to sensitive bacteria from old cultures is considerably less than to sensitive bacteria from young cultures.

The adsorption of all the mutants, and of wild type as well, is very much faster to indicator bacteria grown in a synthetic medium than to indicator bacteria grown in broth. The rates in this instance parallel the efficiencies of plating, which are, on synthetic medium agar: 0.07 for wild type, 0.6 for h^t5, and 1.0 for h^t1 and h^c2.

The distinction between indicator and sensitive bacteria is evidently a relative one in the case of our indicator strain growing in synthetic medium, and the question arises whether the broth cultures and the synthetic medium cultures have the same genetic composition. We do not believe this question can be answered except to say that both kinds of population appear to be homogeneous, and to transform in either direction during a single culture cycle whether started from a massive inoculum or from a single colony. In any case, our data show that the relative susceptibility to different viral mutants is subject to non-heritable as well as heritable modifications in the bacteria.

The very low e.o.p. of the mutant h^t5 on nutrient agar containing peptone is left unexplained except to say that it must depend on conditions in the agar medium which we are unable to reproduce in adsorption measurements. It is perhaps significant that this mutant alone gives a higher plaque count when plated with sensitive bacteria on synthetic medium agar than when plated on agar containing peptone.

Rates of adsorption evidently provide a quantitative measure of mutational effects that is considerably more sensitive than the measurement of e.o.p. The class h^c, for instance, should include all the mutants with rates of adsorption in excess of a certain value, and there is no reason to anticipate that any two of them would be identical. The failure to distinguish h^c1 from h^c2 (table 2) by this method thus adds materially to the evidence that these arose by independent occurrences of the same mutation.

THE PATTERN OF BACK-MUTATION

If the mutant h^c6, arising from h^t1 by a second mutational step, carried alterations at two independently mutating loci, it should be incapable of reverting to the wild genotype in a single step. During serial passage accompanied by loss of the h character, one would expect, among other possibilities, to observe reversion to the phenotype h^t1. If, on the other hand, h^c6 arose by a second mutation in a single mutational locus, so that the second mutation erased the first, reversion to wild type in a single step would be possible, and there should be no preferential selection of the phenotype h^t1 on serial passage. Table 3 shows the patterns of reversion observed when different single-plaque stocks of h^c6 are subjected to serial passage. The behavior of the single-step mutants h^c2 and h^t1 is shown for comparison. The table shows that h^c6 can return to wild type (or to a phenotype resembling wild) in a single mutative step and, more important, that the pattern of reversion of h^c6 is identical to that of the single-step mutant h^c2. We conclude that the muta-

TABLE 3

Serial passage of the mutants h^t1, h^c2, and h^c6.

Series	Percent of turbid plaques on mixed indicator, and analysis of population, after passage number				
	2	4	6	8	10
h^t1 (1)	0	0	1	5(a)	60
h^t1 (2)	0	0	0	5(a)	50
h^t1 (3)	0	0	1	20(a)	50
h^c2 (1)	1	50(b)			
h^c2 (2)	0	50(b)			
h^c2 (3)	0	70(c)			
h^c6 (1)	0	70(c)			
h^c6 (2)	0	50(b)			
h^c6 (3)	0	50(b)			

Each series was started from a different plaque of the specified type. After the indicated number of serial passages, pure lines of phage were isolated from turbid plaques on mixed indicator, and also from turbid plaques, if any, on single indicator, and identified by measuring the e.o.p. The following types of result were obtained in the above and similar experiments:

(a) Mixture of h^t1 and wild type.

(b) Mixture of h^c, h^t, and wild type. The proportion of h^t was usually less than the proportion of wild type, and never exceeded the proportion of h^c before wild type arose. In several instances it was established that the e.o.p. of the isolated h^t (0.2–0.3) was significantly different from that of h^t1.

(c) Mixture of h^c and wild type only. This result was observed in two out of six series starting from h^c2, and three out of seven series starting from h^c6.

tion giving rise to h^c6 has obliterated the previous mutation carried by h^t1, and therefore that these are alternative modifications of a single mutational locus.

In view of the rather complicated pattern of reversion just described, it was decided to repeat the experiment with a two-step mutant derived from a different h^t stock. For this purpose the mutant h^t5, and h^c7 derived from it, were isolated. It was found, quite unexpectedly, that h^c7 reverted during serial passage to h^t5, long before any wild type appeared in the stocks. This behavior points clearly to the conclusion that the two mutations have both left their marks in the stock h^c7, and therefore that at least one of them has occurred in a mutational locus distinct from the site of the mutation h^t1.

It is of some interest that these experiments were done before any crosses had been made with the stocks h^t5 and h^c7, so that the existence of a second locus governing host specificity was discovered by the means just described.

It has happened before (HERSHEY 1946) that the analysis of patterns of back mutation has furnished information about the genetic structure of phage that was later confirmed by other methods. In view of these successes, it is probably unnecessary to enumerate here the several situations in which mutational analysis might suggest erroneous conclusions.

INTERCROSSES AMONG *h* MUTANTS

The results of the following crosses are expressed qualitatively, owing to difficulties in the quantitative recognition of the several viral types. When a recombinant was found, it was always present in the proportion of several percent. The absence of a given recombinant was established by sampling a number of the plaques that resembled it, and identifying the phage in the samples by measuring the e.o.p. Recombinants not found would have been detected by this method if present in a proportion exceeding 0.1 percent.

As previously mentioned, the five indistinguishable mutants of phenotype h^c failed to yield wild type in intercrosses, and the same was true of the crosses $h^c2 \times h^t1$ and $h^c2 \times h^t2$. (The mutants h^t3 and h^t4 could not be distinguished, in terms of e.o.p., from h^t2, and were not used in these experiments.) Evidently all these mutants came from mutations at the same combinative locus, which we shall call locus 1. Our further interest centers on the relation of the mutants h^c6, h^c7, and h^t5 to this locus.

According to the patterns of reversion previously described, the two-step mutant h^c6 is a single-locus mutant identical, so far as can be determined, with the single-step mutant h^c2. The results of the following crosses support this conclusion.

$$h^c6 \times \text{wild type} \longrightarrow \text{no } h^t$$
$$h^c6 \times h^c2 \longrightarrow \text{no wild type}$$

By contrast, the two-step mutant h^c7 behaves like a double mutant, in confirmation of the conclusions reached by analysis of pattern of back-mutation.

$$h^c7 \times \text{wild type} \longrightarrow h^t5 \text{ (e.o.p. 0.01)}$$
$$h^c7 \times h^c2 \longrightarrow \text{no } h^t \text{ or wild type}$$

The last cross shows that the h^c mutation in h^c7 is allelic with h^c2. The following crosses show that h^t5 is not allelic with h^c2 or with h^t1.

$h^t5 \times h^c2 \longrightarrow$ wild type
$h^t5 \times h^t1 \longrightarrow$ wild type and $h^t1\ h^t5$ (e.o.p. 0.5)
$h^t1\ h^t5 \times$ wild type $\longrightarrow h^t1$ (e.o.p. 0.1) and h^t5 (e.o.p. 0.01)

The second (h^c) mutation in h^c7 lies, therefore, in locus 1, and the mutation h^t5 identifies a second combinative and mutative locus.

LINKAGE TESTS

If the conclusions reached above are correct, any phenotype resulting from a mutation in locus 1 ought to recombine with the same frequency as h^c2 in crosses with selected markers, whereas the mutant h^t5 should behave differently. The markers chosen for this test were *r*1, *r*7, *r*13, and *m* (HERSHEY and ROTMAN 1949). The results are shown in table 4. It may be seen that locus 1 of the mutations h^c2, h^c6, and h^t1 is closely linked to *r*13, whereas the locus of the mutation h^t5 is not. The position of this locus is otherwise uncertain, and the methods employed here are unsuited to determine it.

TABLE 4

Linkage relations among h mutants.

	h^c2	h^c6	h^t1	h^t5
*r*1	16–20	15	17	~ 13
*r*7	8–10	9	9	~ 14
*r*13	1–2	1	2	$\lesssim 10$
m	18–20	20	18	~ 10

The numbers indicate percentage yields of wild type in the respective crosses. Yields of recombinants in crosses involving h^t are approximate, because of the difficulty of recognizing the plaques on mixed indicator. The minimal yield of wild type in $h^t5 \times r13$ was established by sampling and confirming 26 typical plaques from two plates showing a total of 231 plaques.

DISCUSSION

It has been shown that a number of host-range mutations of phage T2H can be assigned to one or the other of two loci, identifiable by any one of three independent tests. A number of alternative mutations can occur at one of these loci. The unit character of this locus is established by the failure of successive mutations to accumulate in it, by the inability of its alternative mutations to combine in intercrosses, and by its unique and invariant map position in several mutational states. The second locus, at which only one mutation has been found, is distinguishable from the first by the same three tests. The mutual consistency of these tests gives strong support to the notion of localized hereditary determinants in the phage, and shows further that at least two of the mutations that can occur in a single combinative structure occur at a single mutational site within that structure.

At one of the loci controlling host specificity, a number of mutations can

occur leading sometimes to different visible effects, and sometimes to what appear to be recurrences of the same mutation. This is in contrast to mutations affecting the *r* character, which tend to occur each time at a different locus, as if the number of loci were very great, and their rates of mutation very similar. Neither situation, of course, is without precedent in other organisms.

SUMMARY

Host range mutants of the bacteriophage T2H differ among themselves, and from the wild type, with respect to rate of adsorption to their selective host. These changes occur without affecting the rate of adsorption to the common host. Accompanying these changes, and characteristic of them, are changes resulting in decreased stability of the virus at high temperatures.

Several of the host-range mutants (class 1) fail to give rise to wild type when intercrossed. One host-range mutant (h^t5) yields wild type when crossed with examples of class 1.

A mutant arising in two successive steps, both of class 1, is able to lose its mutant character in a single mutative step. A mutant arising in two mutative steps, the first of which is h^t5, the second of class 1, can lose its mutant character only by two mutative steps, the first of which can yield h^t5 again.

The mutational locus in three mutants of class 1 occupies a single map position with respect to a set of four selected markers. The mutational locus in h^t5 occupies a different position.

We conclude that the inheritance of host specificity in T2H is controlled by genes occupying at least two different loci, and that at least one of these genes is capable of alternative mutations.

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THE INTERACTION OF NUCLEAR AND CYTOPLASMIC FACTORS IN THE INHERITANCE OF MALE STERILITY IN MAIZE *

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MALE sterility in plants is of unusual interest since its inheritance is due to a number of different mechanisms. In the early days of genetics a controversy existed as to the relative importance of the nucleus and the cytoplasm in inheritance. Many investigators believed the nucleus was all-important and relegated the cytoplasm to a non-essential role. That the cytoplasm did not always play an indifferent role in heredity was shown by certain cases of male sterility which were strictly cytoplasmically determined and also by those male steriles resulting from interaction of specific genes and cytoplasm.

Male steriles resulting solely from genic action are most common. EMERSON, BEADLE and FRASER (1935) list twenty different male sterility genes in maize. Gene-determined male steriles have been reported in tomato (CRANE 1915), potato (SALAMAN 1912), barley (SUNESON 1940), and sugar beets (OWEN 1945) as well as in a number of other plants (LEWIS 1941).

BATESON and GAIRDNER (1921) described a male sterile condition in Linum where the phenotype of the F_2 progeny depended upon the direction of the parental cross. When the procumbent race was used as the female parent in crosses with common tall flax, the F_2 population segregated normal and male sterile individuals in a 3:1 ratio. However, the reciprocal cross yielded only normal progeny. CHITTENDEN and PELLEW (1927) and CHITTENDEN (1927) believed these results were due to an interaction between gene and cytoplasm. Plants are sterile only when they possess the cytoplasm of the procumbent race and are homozygous for a specific recessive gene normally found in common tall flax. This interpretation was later confirmed by GAIRDNER (1929).

OWEN (1945) reported a case of male sterility in the sugar beet (*Beta vulgaris*) which also involved gene-cytoplasm interaction. Plants possessing the normal cytoplasm (N) were male fertile while plants with the sterile cytoplasm (S) were either fertile or sterile depending upon their genic constitution. OWEN showed that at least two genes influenced the degree of sterility. Plants with the S cytoplasm and homozygous recessive for both

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genes were completely sterile while those individuals which were heterozygous for one or the other gene were semi-sterile.

JONES and CLARKE (1943) reported a case of male sterility in the onion (*Allium cepa*) which was conditioned by interaction between nuclear and cytoplasmic factors. Male sterility in *Dactylis glomerata* was found to be dependent upon the interaction of the cytoplasm with a dominant gene (MYERS 1946). In the presence of the appropriate cytoplasm plants were male sterile if quadruplex, triplex, or duplex for the sterility gene. They were fertile if nulliplex, and either sterile or fertile if simplex.

EAST (1932) found that certain self-sterility (S) alleles derived from *Nicotiana sanderae* produced male sterile plants if combined with *N. langsdorffii* cytoplasm. However, in cytoplasm derived from *N. sanderae* these same factors produced male fertile plants. EAST further showed that the phenotypic expression of other characters, such as the production of chlorophyll in the corolla, was influenced by the cytoplasmic as well as the genic constitution of the plant.

Cases of cytoplasmically inherited male sterility in maize have been described by JOSEPHSON and JENKINS (1948), and by JONES (1950). The degree of sterility was found to be influenced by contributions from the male parents, indicating interaction with genic factors.

In contrast to the large number of cases of male sterility either gene-determined or due to gene-cytoplasm interaction, there exist rare instances of male sterility where the inheritance is solely cytoplasmic (RHOADES 1933; GABELMAN 1949). This is not surprising since few of the numerous cases described in the literature where a cytoplasmic effect is involved show a strict cytoplasmic heredity, such as found by CORRENS (1928) for gynodioecy in Circium and by L'HERITIER and TEISSIER (1937, 1938) for CO_2 sensitivity in Drosophila. Maternally transmitted chlorophyll defects, due to the inclusion of abnormal proplastids in the egg cytoplasm, are the most common examples of cytoplasmic inheritance. However, considering the universality of gene action and the multitude of effects which have been shown to be gene-determined, it would be surprising if cases of purely cytoplasmic inheritance, other than that of plastids, were anything but rare.

At the present time, RHOADES' male sterile can best be interpreted as a case of purely cytoplasmic inheritance. It was not transmissible through the viable pollen produced by partially sterile plants, and was found to persist even though all of the chromosomes in the sterile line had been replaced by chromosomes known to be free from sterility producing factors. In the event that a gene is found which is able to suppress sterility, this cytoplasmically inherited condition could no longer be considered independent of nuclear control.

It is of interest to note that no cases of sterility have been reported in maize, aside from those due to meiotic irregularities, which affect female as well as male fertility.

The purpose of this paper is to present the analysis of a case of male sterility in maize which is conditioned by an interaction of gene and cytoplasm.

ORIGIN OF MALE STERILE LINE

In connection with a study of a chromosomal aberration involving chromosomes 3 and 5, reciprocal crosses were made between plants 9915 (12) and 9936 (c). Plant 9936 (c) was heterozygous for the aberration and therefore produced some aborted pollen. However, anthers of this plant dehisced normally and shed copious amounts of good pollen. Plant 9915 (12) was a fully fertile plant from the highly inbred line Kys (Kansas Yellow Saline). When progenies from the two reciprocal crosses were grown there was no reason to expect that wholly male sterile individuals would be found. None were found among the offspring from the cross where 9915 (12) was used as the pistillate parent, but the reciprocal cross with plant 9936 (c) as the egg parent segregated fertile and male sterile individuals in a 1:1 ratio (75 fertile : 69 male sterile). The chromosomal aberration segregated normally in both crosses and there was no correlation between the aberration and the male sterile condition (see table 1). The unlike behavior of the reciprocal crosses suggested that some kind of cytoplasmic inheritance might be involved and the material was turned over to the author by DR. RHOADES for further analysis.

TABLE 1

Data showing independent segregation of male sterility and the 3–5 translocation.

Family	Translocation-bearing plants		Translocation-free plants	
	Male sterile	Normal	Male sterile	Normal
10252	4	5	3	8
10555	4	3	6	1
10556	1	2	1	2
Total	9	10	10	11

DESCRIPTION OF MALE STERILITY

With the exception of two partially sterile plants all of the thousands of male sterile plants studied during the course of this investigation showed complete pollen sterility. No viable pollen grains were produced and only rarely were the shriveled anthers exserted from the glumes. There was no female sterility. A study of microsporogenesis revealed that both meiotic divisions in the male sterile plants were normal. Microspore degeneration occurred soon after the completion of the first spore division as was true for the male sterile condition studied by RHOADES. The aborted pollen grains in the shriveled anthers were completely devoid of protoplasmic structures.

THE PROBLEM

Male sterile plants were first found in the progeny from the cross of 9936 (c) × 9915 (12)♂. Family 9936 came from the cross of a translocation-bearing plant with inbred Kys while 9915 was pure Kys. When normal sibs from the cross of 9936 (c) × 9915 (12) were used as pollen parents in mat-

ings with male sterile plants, a ratio of 1 normal : 1 male sterile plant was obtained. Likewise when Kys plants were crossed with male steriles good 1 : 1 ratios were found. If normal sibs from segregating populations were self-pollinated or crossed by Kys, the ensuing progenies consisted solely of normal individuals. The 1 : 1 ratios found in sib crosses of sterile by normal and in crosses of sterile by Kys suggested that a one gene difference was responsible for the expression of male sterility. The difference in the reciprocal crosses indicated that the gene for male sterility was effective only when present in a cytoplasm originally derived from plant 9936 (c). Such interaction of gene and cytoplasm in producing male sterility had been found in other plants and there appeared to be nothing unusual in the genetics of this male sterile. That a more complex genic mechanism was involved became apparent when male sterile individuals were outcrossed to different unrelated lines. To facilitate further discussion these unrelated lines will be referred to collectively as " testers."

The F_1 plants from such outcrosses were invariably normal as were all plants in the F_2 generation. Altogether, 21 lines were tested resulting in 1648 F_1 plants. Not only did male sterile plants fail to appear in the F_2 population, but backcrosses of the F_1 plants to male steriles also failed to segregate for male sterility. Plants of the first backcross generation were again crossed with male sterile plants but only normal offspring appeared. Individuals of the second, of the third, and of the fourth backcross generations were likewise crossed with male sterile plants without recovering any male sterile offspring. This breeding behavior was clearly not in accordance with any simple hypothesis. In contrast to the failure to recover any male sterile segregates in the above crosses, male sterility was found when normal plants of the F_1 and of the various backcross progenies were used as the female parents in crosses with Kys. Obviously Kys carried factors which allowed the expression of male sterility and differed in this respect from all other lines tested.

The ability of Kys to transmit factors for male sterility was tested by crossing Kys with various genetic testers. A series of successive backcrosses to Kys were made in which Kys was the female parent. After five generations of backcrossing a number of plants were crossed with male sterile plants to see if sterility would segregate. No male steriles were found. In another set of experiments Kys was used as the female parent in the first and as the male in the second backcross. When individuals of the first backcross progenies were crossed with male sterile plants the ensuing progenies consisted only of normal plants. However, when individuals of the second backcross population were crossed with male sterile plants, a segregation for normal and male sterile offspring occurred in certain families. Diagrammatically these results are as follows:

$$\text{Male sterile } ♀ \times [\text{Kys} \times \text{F}_1♂] \longrightarrow \text{only normal offspring}$$

$$(1)\quad \text{Male sterile } ♀ \times [(\text{Kys} \times \text{F}_1♂) \times \text{Kys}♂] \begin{cases} \nearrow \text{only normal offspring} \\ \searrow \text{normal and male sterile offspring} \end{cases}$$

It is clear from the results outlined in this section that the inheritance of this male sterile cannot be interpreted on any simple basis.

ANALYSIS OF THE DATA

Male sterile plants first arose in the progeny of the cross 9936 (c) × 9915 (12). Sibs of plant (c) in family 9936 were crossed by plants in family 9915 but the offspring were all male fertile (table 2). It would appear therefore that the sudden appearance of male steriles in the cross involving plant (c) was due to some kind of mutation present in this plant, and evidence will be given that the postulated mutation permitting the expression of male sterility involved a change in the cytoplasm.

TABLE 2

Results from crosses involving Family 9936 and Kys.

Family	Pedigree	Normal plants	Male sterile plants
10247	9936(g) × 9915(12) Kys♂	21	0
10248	9915(11) Kys × 9936(g)♂	4	0
10249	9915(11) Kys × 9936(g)♂	3	0
10250	9915(9) Kys × 9936(f)♂	15	0
10251	9936(f) × 9915(15) Kys♂	14	0
10252	9936(c) × 9915(12) Kys♂	75	69
10253	9915(12) Kys × 9936(c)♂	25	0
10254	9915(12) Kys × 9936(c)♂	21	0

If cytoplasmic inheritance alone were involved, all of the F_1 progeny of a male sterile plant would be sterile. This obviously is not the case since male steriles when crossed with testers and with Kys yielded F_1 populations consisting of all normal and of half normal and half male sterile plants, respectively.

The good 1 : 1 backcross ratios obtained in the F_1 of crosses of male steriles with Kys clearly indicate that a single gene difference is involved (table 3). Inasmuch as Kys is a highly inbred line and all Kys plants gave the same results when used in crosses, it may be assumed that this strain is homozygous for the factor concerned in the determination of male sterility. Thus, the male sterile plants would be heterozygous for a dominant sterility factor and the crosses with Kys could be regarded as backcrosses to the homozygous recessive. This gene has been designated Ms_{21} (the subscript will be omitted in subsequent references to this gene in the rest of the paper). The male sterile plants would then be genotypically *Msms*, while Kys would be homozygous for the recessive allele, *ms*.

On this basis, the normal plants arising from a cross of male steriles by Kys are genotypically similar to Kys. They differ in that the former possess a cytoplasm which, it is believed, is essential for the expression of the male sterile condition. This hypothesis is substantiated by the fact that male steriles

TABLE 3

Data showing the 1:1 segregation resulting from crosses of male steriles by Kys.

Family	Pedigree Male sterile × Kys♂	Normal	Male sterile
10783	10252(4) × Kys(14)	58	47
D-32	10784(3) × Kys(19)	58	67
D-115	D-30(3) × D-17(1)	47	44
D-605	D-108(6) × D-133(6)	22	20
D-639	D-116(5) × D-133(8)	39	37
D-641	D-115(1) × D-133(25)	24	21
D-648	D-114(3) × D-133(8)	84	60
D-661	D-119(6) × D-133(10)	44	42
D-675	D-119(25) × D-133(6)	76	74
D-682	D-121(3) × D-133(10)	27	30
D-685	D-121(6) × D-133(25)	54	42
D-705	D-124(5) × D-133(14)	48	36
D-723	D-127(11) × D-133(21)	14	17
D-739	11617(22) × D-133(6)	79	67
D-752	11618(7) × D-133(11)	23	28
	Total	697	632

pollinated by their normal sister plants also segregate for sterility in a 1:1 backcross ratio (table 4). No male steriles would be expected in the progeny of self-pollinated normal plants. None were found. Crosses of these normal plants with Kys also failed to yield male steriles in the progenies.

It is the behavior of the male sterile plants in crosses with strains of maize

TABLE 4

Data showing the 1:1 ratios resulting from crosses of male steriles by normal siblings.

Family	Pedigree Male sterile × Normal sib♂	Normal	Male sterile
D-30	10781(8) × 10781(14)	54	66
D-31	10781(16) × 10781(7)	161	167
D-37	10791(5) × 10791(7)	39	41
D-119	D-37(1) × D-37(2)	46	46
D-121	D-39(2) × D-39(5)	45	43
D-126	10792(21) × 10792(15)	43	41
D-607	D-108(4) × D-108(2)	89	78
D-640	D-116(6) × D-116(7)	74	80
D-644	D-114(1) × D-114(8)	59	58
D-652	D-114(7) × D-116(9)	66	77
D-667	D-119(16) × D-127(5)	67	63
D-673	D-119(23) × D-127(5)	62	76
D-674	D-119(24) × D-119(14)	84	68
D-694	D-121(20) × D-121(3)	47	59
D-720	D-127(5) × D-103(4)	76	82
D-725	D-127(20) × D-124(1)	67	75
D-736	11617(13) × 11617(12)	47	40
	Total	1126	1160

other than Kys which reveals that something more than a simple interaction between gene and cytoplasm is involved. The F_1 populations resulting from such crosses were composed only of normal plants. Regardless of whether or not the tester lines carried the dominant *Ms* allele, sterile plants should have been obtained in the F_1. This is shown below where all possible constitutions of the male parent are given.

(2)

Male sterile (*Msms*) × Tester strain♂

If
- *MsMs* ⟶ all male sterile
- *Msms* ⟶ 3 male sterile : 1 normal
- *msms* ⟶ 1 male sterile : 1 normal

Since no sterile plants were found, it could be argued that the tester lines carried a third allele, *Ms′*, dominant over the other two. Its presence in the genome would result in the expression of the normal phenotype. This would explain the absence of sterility in the F_1 population.

(3)

Male sterile (*Msms*) × Tester strain♂ (*Ms′Ms′*)

↓

Ms′Ms—normal
Ms′ms—normal

According to this scheme, sterility should be recovered when F_1 plants are backcrossed to male steriles. Eggs fertilized by sperm carrying the *Ms′* allele would be normal, but eggs fertilized by *Ms* or *ms* sperm, with the exception of *msms* zygotes, would produce male sterile plants.

Similarly, the F_1 hybrids of outcrossed Kys should segregate sterility when crossed with male sterile plants as is shown below:

(4)

Kys (*msms*) × Tester strain♂ (*Ms′Ms′*)

↓

Male sterile (*Msms*) × F_1♂ (*Ms′ms*) ⟶
- *Ms′Ms*—normal
- *Ms′ms*—normal
- *Msms*—male sterile
- *msms*—normal

Inasmuch as all of the offspring from these crosses were normal, it is obvious that a scheme involving a third allele is inadequate.

The failure to find male sterile plants in the F_1 from crosses of male steriles by testers can be accounted for if the male parents were homozygous for a dominant gene which inhibited the expression of male sterility. This suppressor locus will be designated by the symbol *S*. Since Kys differed from the tester lines in permitting the expression of sterility in the F_1, it must be homozygous for the *s* allele, as must also be the male sterile plants.

The unlike behavior of reciprocal crosses involving male fertile plants derived from the crosses of male sterile individuals with the testers is illumi-

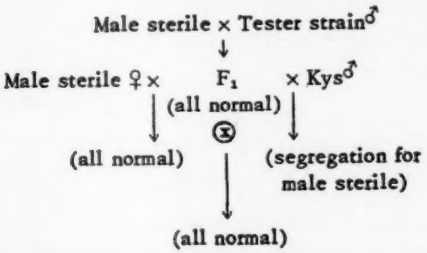
TABLE 5

Breeding behavior of male sterile—tester hybrids.

Family	Pedigree	Normal	Male sterile
D-1229	D-545(5) × D-539(5)N sib♂	54	65
D-1231	D-550(1) × D-539(23)N sib♂	20	5
D-1232	D-550(2) × D-539(5)N sib♂	32	35
D-1233	D-550(4) × D-539(23)N sib♂	40	34
D-1234	D-551(3) × D-539(23)N sib♂	42	12
D-1235	D-551(4) × D-539(23)N sib♂	19	6

nating. When F_1 plants were crossed with male steriles there was no recovery of sterility. Individuals from the first backcross progeny were likewise crossed with male steriles but no sterile plants were found in the second backcross populations. Plants from four successive backcross populations were tested in crosses with male sterile individuals but the invariable result was that no steriles were found. Self-pollinations of F_1 plants, or of individuals in the various backcross progenies, failed to yield sterile offspring. On the other hand, when plants of the F_1 or different backcross progenies were used as the egg parents in crosses with either Kys or normal siblings from segregating populations, a segregation of male steriles occurred (tables 5 and 6). The results from the above crosses may be diagrammatically represented as follows:

(5)



To return to the suppressor hypothesis, the F_1 plants from the cross of male sterile by tester lines would be genotypically *Ss* and therefore male fertile. Certain of the F_2 progenies (depending on the *Ms* constitution) from self-pollinated F_1 plants should segregate for sterility, but this was not the case. Likewise, F_1 plants backcrossed to male steriles unexpectedly produced all normal offspring. It was only when the *Ss* plants were pollinated by Kys (*ss*) that the *ss* constitution allowing the expression of sterility was recovered.

TABLE 6

Summary of progenies derived from backcrossed male sterile-tester hybrids pollinated by Kys.

1 Normal : 1 Male sterile segregating progeny	3 Normal : 1 Male sterile segregating progeny	All Normal progeny
11	17	9

Evidently no segregation for the suppressor factor occurred when the F_1 plants were used as male parents. This becomes understandable when we assume that the postulated *S* locus was closely linked with or functioned as a gametophyte factor, which effected differential fertilization in competition with s pollen. Moreover, the elimination of *s* gametes was complete, since not a single male sterile plant appeared.

If the suppressor-gametophyte complex were composed of two separable genes, *S* and *Ga*, one would expect to find occasional sterile plants in later generations of outcrossed male steriles. A crossover occurring between the two loci would give rise to a gamete, *s Ga*, in which the recessive suppressor gene was linked with the dominant gametophyte factor. Such a gamete would function no differently from *S Ga* pollen. Ovules fertilized by the *s Ga* gametes, being homozygous for the recessive *s* gene, would develop into male sterile plants. However, among 2300 plants resulting from such crosses no male steriles were recovered. Since we cannot as yet distinguish between one gene with two effects and two closely linked loci, we will designate the suppressor locus by the symbol S^{Ga}.

Thus far, three factors have been invoked in explaining the inheritance of the male sterile: (1) a suitable cytoplasm derived from plant 9936 (c), (2) a dominant gene (*Ms*) producing male sterility when combined with the proper cytoplasm, and (3) a suppressor locus associated with a gametophyte effect. The inferred constitutions of the various lines described are as follows:

Kys plants—○ $msms\ s^{Ga}s^{Ga}$

* Male steriles—□ $Msms\ s^{Ga}s^{Ga}$

Normal sibs of male sterile plants—□ $msms\ s^{Ga}s^{Ga}$

Plants used in outcrosses—○ $MsMs\ S^{Ga}S^{Ga}$

(□ represents the cytoplasm necessary for sterility while ○ designates normal cytoplasm.)

According to the scheme outlined above, a 1 : 1 ratio for sterility would be obtained when a male sterile plant was pollinated either by a normal sib or by Kys, due to the segregation of the *Msms* genes. When the male sterile plants were crossed with the testers, the progenies would be all normal because of the presence of the suppressor factor.

(6)

Male sterile (□ $Msms\ s^{Ga}s^{Ga}$) × Tester strain♂ (○ $MsMs\ S^{Ga}S^{Ga}$)

↓

F_1

□ $MsMs\ S^{Ga}s^{Ga}$—normal

□ $Msms\ S^{Ga}s^{Ga}$—normal

* The male sterile plants are represented as heterozygous for the *Ms* gene since they arose only from crosses involving Kys and thus must carry the recessive *ms* allele.

Considering only the S^{Ga} locus, the F_1 plants would form two types of gametes in equal numbers, but pollen grains with the recessive allele, s^{ga}, would not compete successfully with S^{Ga} pollen. Thus, when F_1 plants were crossed with male steriles, only S^{Ga} gametes functioned and suppression of sterility resulted.

(7)

Male sterile × F_1♂
(□ $s^{ga}s^{ga}$) ($\square\ S^{Ga}s^{ga}$)

gametes (s^{ga}) and gametes (S^{Ga}), (s^{ga})—cannot function in competition with S^{Ga}

□ $S^{Ga}s^{ga}$—normal

The heterozygous $S^{Ga}s^{ga}$ constitution persisted through a series of backcrosses to male sterile plants and consequently sterile offspring were never recovered. Similarly, when individuals of the F_1 and backcross generations were self-pollinated only gametes carrying S^{Ga} effected fertilization, and all of the offspring were normal.

The behavior of heterozygous $S^{Ga}s^{ga}$ plants used as female parents is readily explained by the proposed hypothesis. Since the gametophyte factor governs pollen tube growth and does not adversely affect the female gametophyte, half of the functional ovules carry S^{Ga} and half s^{ga}. The s^{ga} ovules may or may not give rise to male sterile plants, depending on the presence or absence of the sterility gene (*Ms*), but those ovules which carry the dominant gene S^{Ga} produce only normal plants.

Three types of ratios of normal to male sterile plants would be expected when $S^{Ga}s^{ga}$ plants are used as female parents in crosses with Kys and normal sibs of male steriles. A 1 : 1 ratio would be obtained from a cross in which the female is homozygous *Ms*.

(8)

□ *MsMs* $S^{Ga}s^{ga}$ × *msms* $s^{ga}s^{ga}$♂

↓

□ *Msms* $S^{Ga}s^{ga}$—normal
□ *Msms* $s^{ga}s^{ga}$—male sterile

A 3 : 1 ratio would be expected when the female parent is heterozygous *Msms* as in the following cross:

(9)

□ *Msms* $S^{Ga}s^{ga}$ × *msms* $s^{ga}s^{ga}$♂

↓

□ *Msms* $S^{Ga}s^{ga}$—normal—due to suppressor gene
□ *Msms* $s^{ga}s^{ga}$—male sterile
□ *msms* $S^{Ga}s^{ga}$—normal—lacks sterility gene
□ *msms* $s^{ga}s^{ga}$—normal—lacks sterility gene

Crosses involving plants homozygous recessive *ms* would yield all normal progenies.

(10)

$$\square\ msms\ S^{Ga}s^{ga} \times msms\ s^{ga}s^{ga}\ ♂$$
$$\downarrow$$
$$\square\ msms\ S^{Ga}s^{ga}\text{—normal}$$
$$\square\ msms\ s^{ga}s^{ga}\text{—normal}$$

Ratios of all three types have been observed. Since the F_1 plants of male sterile by tester hybrids are either homozygous or heterozygous for the dominant sterility gene, all progeny resulting from crosses of these plants as the female parents by Kys or normal sibs of male steriles should segregate sterility in either 1 : 1 or 3 : 1 ratios. No all normal progeny could arise (table 5). However, if the hybrid is backcrossed to male steriles before being used in the cross with Kys, all three types of progeny can result (table 6).

Normal plants resulting from the crosses shown in diagrams 8, 9, and 10 were subsequently crossed with male sterile plants. On the basis of the proposed hypothesis it should be possible to predict the results of such crosses.

The normal plants from 1 : 1 segregating populations (diagram 8) are heterozygous for the suppressor factor and hence should yield only normal progeny. No sterile plants resulted in the progeny of eight such crosses.

The normal plants in diagram 9 which are homozygous for the recessive suppressor (s^{ga}) are also homozygous recessive *msms*. Thus any resulting progeny which segregate sterility should do so in a 1 : 1 ratio. Unfortunately only one such cross was made but it conformed with the prediction, yielding half normal and half sterile offspring.

The normal plants in diagram 10 are of two types, heterozygous and homozygous recessive for the suppressor factor. The former should yield only normal progeny when crossed with male steriles, while the latter should produce half normal and half sterile plants. Five normal plants were used; three segregated sterility in a 1 : 1 ratio while the remaining two gave all normal progeny.

Normal plants from 1 : 1 segregating populations (diagram 8) are heterozygous for both factors and hence should give progenies consisting of ¾ normal and ¼ sterile offspring when used as the female parents in crosses with Kys. This too was found to be the case.

The complete correspondence between the observed and predicted results strengthens the validity of the hypothesis.

Further evidence supporting the gamete factor hypothesis may be derived from an analysis of the behavior of Kys hybrids. Kys was outcrossed and the hybrid subsequently backcrossed to Kys for five generations. In all of these crosses Kys was the female parent. The B_5 plants should be 98.5 percent pure Kys but in crosses with male steriles they behaved like the tester plants in that only male fertile plants resulted. Since only Kys cytoplasm was involved in the backcrosses, the difference in behavior of B_5 and Kys plants must be

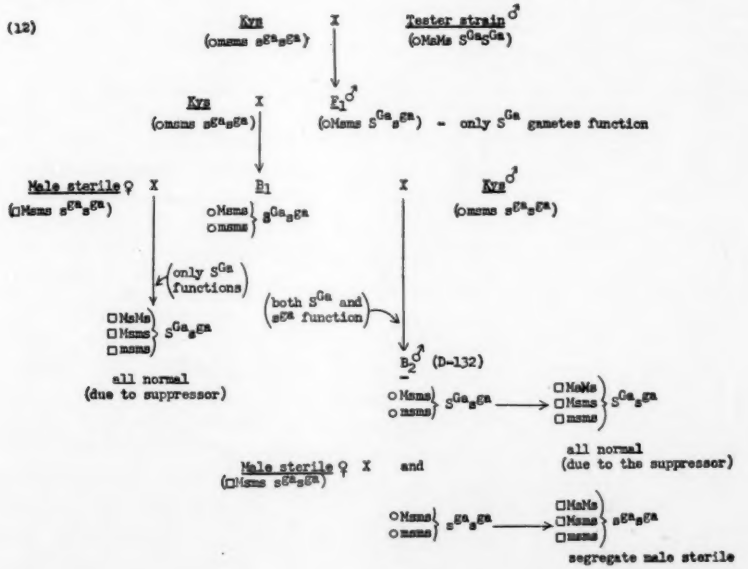
genic. After testing six series of backcrosses it became obvious that selective fertilization was operating and that a gene suppressing sterility had not been eliminated. On the basis of the S^{Ga} scheme it would be expected that the suppressor gene would persist indefinitely through a series of backcrosses. This can be represented schematically in the following manner:

(11)

Kys $\times$ Tester strain♂
$(s^{Ga}s^{Ga})$ | $(S^{Ga}S^{Ga})$
↓
Kys $\times$ F_1♂
$(s^{Ga}s^{Ga})$ | $(S^{Ga}s^{Ga})$—only S^{Ga} gametes function
↓
Kys $\times$ B_1♂
$(s^{Ga}s^{Ga})$ | $(S^{Ga}s^{Ga})$—only S^{Ga} gametes function
↓
etc.

In another experiment Kys was used as the female parent in the first backcross (B_1) and as the male in the second backcross (B_2). The cross of the B_1 individuals with male steriles should yield no sterile plants since only S^{Ga} gametes would function in fertilization and all of the offspring would carry the suppressor. However, sterility should be recovered in the progeny of the B_2 cross since only half of the offspring would carry S^{Ga} (see diagram 12). These are precisely the results which were obtained. The B_1 crosses yielded

(12)


Kys (○msms $s^{Ga}s^{Ga}$) X Tester strain♂ (○MsMs $S^{Ga}S^{Ga}$)
Kys (○msms $s^{Ga}s^{Ga}$) X F_1♂ (○Msms $S^{Ga}s^{Ga}$) - only S^{Ga} gametes function
Male sterile♀ (□Msms $s^{Ga}s^{Ga}$) X B_1 ○Msms ○msms $s^{Ga}s^{Ga}$ X Kys♂ (○msms $s^{Ga}s^{Ga}$)
only S^{Ga} functions
both S^{Ga} and s^{Ga} function
□MsMs □Msms □msms $S^{Ga}s^{Ga}$
all normal (due to suppressor)
B_2♂ (D-132)
○Msms ○msms $S^{Ga}s^{Ga}$ → □MsMs □Msms □msms $S^{Ga}s^{Ga}$
all normal (due to the suppressor)
Male sterile♀ (□Msms $s^{Ga}s^{Ga}$) X and
○Msms ○msms $s^{Ga}s^{Ga}$ → □MsMs □Msms □msms $s^{Ga}s^{Ga}$
segregate male sterile

only normal plants while the B_2 crosses produced all normal and segregating families (table 7).

The data support the argument that the expression of male sterility is conditioned by an interaction between the cytoplasm and two genes, *Ms* and s^{ga}. The evidence for cytoplasmic inheritance will be presented in a separate section. However, an examination of the series of crosses in diagram 12 is revealing. Some of the B_2 plants had a genetic constitution required for male sterility, $Msms\ s^{ga}s^{ga}$, but were nevertheless normal. These plants were male fertile because they lacked the specific cytoplasm necessary for sterility. Sterility resulted when this genotype was combined with the proper cytoplasm.

TABLE 7

Summary of data showing the divergent behavior of converged Kys lines from different systems of breeding in crosses with male sterile plants.

Family	Pedigree Male sterile × Kys hybrid♂	Pedigree of Kys hybrid*	Normal	Male Sterile
D-610	D-108(7) × D-132(12)	$[A_1 \times (A \times B)♂] \times A_1♂$	10	5
D-656	D-119(1) × D-132(12)	"	27	30
D-657	D-119(2) × D-132(12)	"	21	12
D-658	D-119(3) × D-132(12)	"	5	4
D-659	D-119(4) × D-132(12)	"	21	16
D-684	D-121(5) × D-132(12)	"	25	13
D-689	D-121(13) × D-132(12)	"	18	14
D-672	D-119(21) × D-132(9)	"	41	52
D-550	D-127(14) × D-132(4)	"	20	0
D-687	D-121(10) × D-131(7)	$A_1 \times (A \times B)♂$	25	0
D-698	D-122(1) × D-131(11)	"	22	0
D-1109	D-544(2) × D-569(2)	$A_3 \times (A \times B)♂$	77	0
D-1112	D-539(67) × D-572(1)	"	16	0
D-1114	D-539(24) × D-558(1)	"	15	0
D-1118	D-539(9) × D-561(3)	"	28	0
D-1122	D-539(48) × D-567(1)	"	11	0
D-1123	D-539(28) × D-564(1)	"	13	0
D-1124	D-539(21) × D-564(2)	"	22	0

* A = Kys
B = Tester strain

PARTIAL STERILITY

Thousands of male sterile plants were observed in the course of the investigation and with only two exceptions all were completely pollen sterile. The two exceptional plants occurred in family 10252 [9936 (c) × 9915 (12)♂] which consisted of 75 male fertile : 67 wholly male sterile : 2 partially male sterile individuals. The tassels of the partially sterile plants were sectorial for fertile and sterile regions. It seemed likely that these plants were heterozygous S^{Ga}/s^{ga} and that somatic elimination of the S^{Ga} allele had occurred during tassel development. A partially sterile plant was pollinated by Kys; the progeny consisted of 30 fertile : 11 completely male sterile individuals (a 3 : 1 ratio). Four of the eleven sterile plants were crossed by two normal sibs. Their progeny contained no male sterile plants in striking

contrast to the 1 : 1 ratios found in all other sib crosses of sterile by normal. Further crossing of these normal individuals to male steriles from families with a ratio of 1 fertile : 1 sterile failed to yield male sterile offspring, *i.e.*, their breeding behavior was identical with that of the tester lines. Individuals of the fourth backcross population (B_4), when self-pollinated, again produced only normal offspring. However, a B_4 plant used as a female parent in a cross with a *msms* $s^{ga}s^{ga}$ plant gave a 1 : 1 segregation in the progeny.

These results are consistent with the hypothesis already advanced if it is assumed that the partially male sterile plant was heterozygous for the suppressor gene. The validity of this assumption is evident from an analysis of the pedigree of family 10252. In the following diagram the inferred constitution is given in parentheses.

9024 (2) (*MsMs* $S^{Ga}S^{Ga}$) male fertile × Kys♂ (*msms* $s^{ga}s^{ga}$) male fertile ⟶ 9589 (*Msms* $S^{Ga}s^{ga}$) all male fertile

(13)

9589 (11) (*Msms* $S^{Ga}s^{ga}$) male fertile × 9599 (17)♂ (*MsMs* $S^{Ga}S^{Ga}$) male fertile ⟶ 9936 (*MsMs* $S^{Ga}S^{Ga}$), (*Msms* $S^{Ga}S^{Ga}$), (*MsMs* $S^{Ga}s^{ga}$), (*Msms* $S^{Ga}s^{ga}$) all male fertile

9936 (c) (*MsMs* $S^{Ga}s^{ga}$) male fertile × 9915 (12)♂ (*msms* $s^{ga}s^{ga}$) male fertile ⟶ 10252 (*Msms* $S^{Ga}s^{ga}$—normal), (*Msms* $s^{ga}s^{ga}$—male sterile)

All of the individuals in family 10252 possessed the *Ms* allele necessary for sterility, but in half of the plants the S^{Ga} factor was heterozygous and the *Ms* action was suppressed. Loss of the S^{Ga} gene in the cells of the developing tassel would result in sterile sectors, as is thought to be the case in the two partially sterile plants.

The breeding behavior of the partially sterile plant now becomes clear. A 3 : 1 ratio would be expected in a cross to Kys since both *Ms* and S^{Ga} were segregating:

10252 (21) Part. male sterile (□ *Msms* $S^{Ga}s^{ga}$) × Kys♂ (○ *msms* $s^{ga}s^{ga}$)

(14)

10786

□ *Msms* $S^{Ga}s^{ga}$—normal (type 1)
□ *Msms* $s^{ga}s^{ga}$—male sterile
□ *msms* $S^{Ga}s^{ga}$—normal (type 2)
□ *msms* $s^{ga}s^{ga}$—normal (type 3)

No male steriles were recovered when sterile plants from the above cross were mated with two of the normal sibs. Thus, the normal sibs must have been of type 1 or type 2 (see above) and produced only functioning S^{Ga} pollen. Continued backcrossing of the resulting fertile plants to male steriles failed to produce sterile offspring. However, after four generations of backcrossing, a B_4 plant used as female with a *msms* $s^{ga}s^{ga}$ male segregated sterile and normal offspring in a 1 : 1 ratio (see below).

(15)

B_4 (□ *MsMs* $S^{Ga}s^{ga}$) × Normal sib of ♂ male sterile (□ *msms* $s^{ga}s^{ga}$)

↓

D-1147

□ *Msms* $S^{Ga}s^{ga}$—normal

□ *Msms* $s^{ga}s^{ga}$—male sterile

The backcrosses were made to male sterile plants heterozygous for the sterility gene and therefore some of the progeny were homozygous recessive *ms*. A B_2 plant used as a female parent in a cross with Kys was evidently of this constitution (*msms*) since only normal plants were found in the progeny (D–548). Further testing showed that these plants were normal not because of the presence of the suppressor but due to the absence of the sterility gene. When D–548 was the female parent in crosses with normal sibs of male steriles and Kys, only normal offspring were produced. However, segregation for male sterility occurred in 1 : 1 ratios when D–548 was used as the male parent in crosses with male steriles. Therefore, D–548 plants did not carry S^{Ga} and were male fertile due to the absence of the *Ms* gene.

(16)

Male sterile ♀ (□ *Msms* $s^{ga}s^{ga}$) × D-548 (□ *msms* $s^{ga}s^{ga}$) × Normal sib of ♂ male sterile (□ *msms* $s^{ga}s^{ga}$)

↓ ↓

□ *Msms* $s^{ga}s^{ga}$—male sterile

□ *msms* $s^{ga}s^{ga}$—normal

□ *msms* $s^{ga}s^{ga}$—normal

THE CYTOPLASMIC FACTOR

Cytoplasmic inheritance is commonly manifested by the dissimilar appearance of reciprocal hybrids, but the occurrence of unlike hybrids is not in itself convincing evidence of a specific cytoplasmic quality. If the genic contributions by the two parents can be shown to be equal and if such phenomena as predetermination and dauermodifications can be proved non-existent, only then it is permissible to assign persisting differences in reciprocal crosses to the greater cytoplasmic contribution of the egg parent. However, reciprocal crosses are impossible where male sterility is involved and evidence for a

cytoplasmic factor in the inheritance of male steriles must come in other ways. A further difficulty encountered in this study is the selective fertilization produced by the S^{Ga} allele.

The mode of inheritance of the male sterile condition appears comprehensible only if a cytoplasmic factor is involved. Plants of identical genic constitutions are male fertile or male sterile depending upon the cytoplasm contributed by the female parent. However, before accepting the validity of this interpretation all other hypotheses must be negated. What are some of these alternative mechanisms and why do they fail?

According to the proposed hypothesis some of the B_2 Kys backcross plants (see diagram 12) contained the proper genotype for sterility but were normal. Any alternative hypotheses not invoking the cytoplasm must assume a genic difference between these B_2 plants and the male sterile individuals. The male sterile condition would thus be due to the presence of a third allele at the *ms* locus. On this basis, the alleles found in tester, male sterile, and Kys plants could be designated *Ms′*, *Ms*, and *ms* respectively.

There are two alternative hypotheses to be considered: (1) *Ms′* and S^{Ga} are one and the same gene and (2) *Ms′* and S^{Ga} represent two different loci as has previously been postulated. According to (1) the *Ms′* allele would show dominance over *Ms* in that the heterozygous *Ms′/Ms* plants would be male fertile. The F_1 hybrids of male steriles by testers should segregate sterility in a 1 : 1 ratio or yield all normal progeny when crossed with Kys. No progenies segregating in a ratio of 3 normal : 1 male sterile would be obtained from such crosses. This is shown by the following diagram:

(17)

Male sterile × **Tester strain**♂
(Msms) | *(Ms′Ms′)*
↓
F_1 (Kys)♂
Ms′Ms × *msms* ⟶ { *Ms′ms*—normal; *Msms*—male sterile (1 : 1)

and

Ms′ms × *msms* ⟶ { *Ms′ms*—normal; *msms*—normal (all normal)

Furthermore, when the F_1 hybrid has been backcrossed to male steriles and the B_1 used as female parents in crosses with Kys, no 3:1 ratio should result as is shown below:

(18)

Male sterile × F_1♂
Msms | *Ms′Ms* (*ex hypothesis* only *Ms′* effects fertilization)
Ms′ms

B_1 (Kys)♂
Ms′Ms × *msms* ⟶ {*Ms′ms*—normal; *Msms*—male sterile} (1:1)

and

Ms′ms × *msms* ⟶ {*Ms′ms*—normal; *msms*—normal} (all normal)

The data obtained do not support this hypothesis. Ratios of 3 normal : 1 male sterile were found in the progeny of the F_1 and various backcrosses (tables 5 and 6).

According to the second alternative only 3:1 ratios and all normal progenies would be expected from crosses of the F_1 hybrids as females with Kys.

(19)

Male sterile × Tester strain♂
(Msms $s^{ga}s^{ga}$*)* | *(Ms′Ms′* $S^{Ga}S^{Ga}$*)*

F_1 (Kys)♂
Ms′Ms $S^{Ga}s^{ga}$ × *msms* $s^{ga}s^{ga}$ ⟶ {*Ms′ms* $S^{Ga}s^{ga}$—normal; *Ms′ms* $s^{ga}s^{ga}$—normal; *Msms* $S^{Ga}s^{ga}$—normal; *Msms* $s^{ga}s^{ga}$—male sterile} (3:1)

and

Ms′ms $S^{Ga}s^{ga}$ × *msms* $s^{ga}s^{ga}$ ⟶ {*Ms′ms* $S^{Ga}s^{ga}$—normal; *Ms′ms* $s^{ga}s^{ga}$—normal; *msms* $S^{Ga}s^{ga}$—normal; *msms* $s^{ga}s^{ga}$—normal} (all normal)

The fact that 1:1 ratios were obtained from such crosses shows that this scheme will not adequately explain the data (table 5).

Additional evidence that this alternative is invalid may be derived from an analysis of the pedigree of family 10252 (see diagram 13). Plant 9936 (c) was heterozygous for the suppressor gene. Since family 10252 segregated

male sterility in a 1 : 1 ratio, this plant must also have been homozygous for the *Ms* allele which causes sterility. This could be so only if the allele present in the male sterile plants was the same as that in the tester strains. Thus, the alternative hypothesis involving a third allele cannot be maintained. Cytoplasmic inheritance readily explains why Kys-tester hybrids are not sterile even though they possess the proper genotype.

NEW MALE STERILE

A new male sterile condition arose spontaneously in the progeny of a Kys-tester hybrid. It is completely male sterile and has no detectable effect on female fertility. Pollen abortion results from a degeneration of the microspores following the first mitotic division. Its breeding behavior is identical with that of the male sterile found earlier in family 10252.

The origin of this new male sterile is as follows: Three plants of family D–132 were used as females in crosses with Kys. Two of the crosses yielded only normal offspring while the third segregated 76 normal to 62 male sterile plants (D–544). One of these male sterile plants was pollinated by Kys and segregated for sterility in a 1 : 1 ratio (50 normal : 66 male sterile). A second male sterile plant was crossed with a normal sib from the original sterile line and its progeny consisted of 14 normal and 8 male sterile plants. Crosses of normal plants in family D–544 with Kys produced only normal offspring. The most striking resemblance between the two male sterile conditions is shown in crosses of the new male steriles with Kys-tester hybrids which had been backcrossed to Kys for five generations. These crosses yielded only normal offspring, in conformity with the behavior of the original male sterile line.

In accordance with the hypothesis which has been proposed, some plants in family D–544 contained the genotypic constitution but not the proper cytoplasm for male sterility, since the cytoplasm was derived from Kys. The appearance of male sterile plants in family D–544 is attributed to a mutation of the cytoplasm. It is possible to determine the precise plant in which the mutation arose. Family D–132 presumably was composed of plants with four possible genotypes, (1) $Msms\ S^{Ga}s^{ga}$, (2) $Msms\ s^{ga}s^{ga}$, (3) $msms\ S^{Ga}s^{ga}$, (4) $msms\ s^{ga}s^{ga}$ (see diagram 12). The genic constitution of plant D–132 (2), which gave rise to family D–544, was of type (2) since a 1 : 1 ratio of normal to male sterile plants was found. The fact that this plant was normal, although it possessed the proper genic constitution for male sterility and produced some male sterile offspring, indicates that the cytoplasmic mutation must have occurred in plant D–132 (2).

In the case of the original male sterile it was not possible to ascertain the precise time at which the mutation to the " sterile " cytoplasm occurred. Although male sterile plants first appeared in family 10252, the mutation could have occurred earlier but was not detected since the parental plants were not of the proper genotype for the expression of male sterility. As is evident from the pedigree of family 10252 (see diagram 13), all plants used as females were

either homozygous or heterozygous for S^{Ga}. The obvious conclusion is that plant 9936 (c) possessed the "sterile" cytoplasm but was not necessarily the site of the mutation.

DISCUSSION

The genetic mechanism for the inheritance of the male sterile condition involves three factors: (1) a specific cytoplasm, (2) a dominant gene (*Ms*) for sterility, and (3) a dominant suppressor (S^{Ga}) associated with a gametophyte effect. As has been pointed out, the dual effect of S^{Ga} may be the result of two closely linked loci. There is individually nothing exceptional in the behavior of the three factors, but their interaction in producing the male sterile condition results in a genetic situation of some complexity.

Owing to the unusual inheritance of the S^{Ga} factor, which results in an apparent absence of segregation in heterozygous plants, little progress has been made in the study of this gene. However, three methods are being employed which should prove fruitful. (1) If a sparse pollination is made so that one or few pollen grains lodge on each style there would be little or no competition between S^{Ga} and s^{Ga} pollen. Some s^{Ga} sperm should effect fertilization thus giving rise to male sterile individuals. (2) Treatment of S^{Ga} pollen with X-rays should result in male sterile plants. Since both mitotic divisions of the male gametophyte in maize occur before the pollen is shed, a mature grain contains the tube nucleus and two sperm cells. Germination and growth of a pollen tube is governed by the tube nucleus. If the X-ray "hit" should delete the S^{Ga} locus from a sperm cell while the tube nucleus remained unaffected, the growth of the pollen tube would be normal. Such a tube could discharge a sperm deficient for the S^{Ga} gene into the embryo sac, and in the eventuality that this sperm fertilized the egg cell a male sterile individual would result. (3) The gametophyte effect of S^{Ga} can be used to ascertain its location in the chromosomal complement. All tester stocks studied are $MsMs\,S^{Ga}S^{Ga}$ while Kys is $msms\,s^{Ga}s^{Ga}$. If tester stocks marked with mutant genes for all of the ten chromosomes are crossed with Kys and the F_1 hybrids used as males in backcrosses to the tester strains, normal 1 : 1 ratios will be obtained except for those loci which lie in the same chromosome as the S^{Ga} locus. The deviation from a 1 : 1 ratio will depend upon the amount of recombination between S^{Ga} and the marker gene.

In view of the admitted complexity of the hypothesis advanced to account for the inheritance of the suppressor factor, any alternative must be considered. One possible explanation is worthy of comment although it does not appear to be consistent with the data. MAMPELL (1946) believed that mutation inducing particles in Drosophila arose when a recessive autosomal gene was present with a Y chromosome. These mutator particles were transmitted by the sperm cytoplasm but not by the egg cytoplasm unless the female was XXY. It might be argued that the tester strains in maize contain cytoplasmic particles capable of suppressing male sterility and that these particles are transmitted through the sperm but not through the egg. This scheme would account for the differences of the reciprocal backcrosses of Kys-tester hybrids,

but would not explain the results obtained from the following series of crosses:

(20)

Kys × Tester strain♂
↓
Kys × F_1♂
↓
B_1 × Kys♂
↓
Male sterile × B_1♂
↓
3 families with 1 male sterile : 1 normal
1 family with all normal

The plants in the B_2 population should not possess the cytoplasmic particles since they arose from a cross where Kys was used as the male parent. However, one of the B_2 plants carried the suppressor since its progeny from a cross with male sterile consisted solely of normal offspring. Other data likewise negate the idea that the suppressor action is due to cytoplasmic particles transmitted by the sperm.

The gametophytic component of the S^{Ga} locus is not unique in its activity. A number of cases have been reported in the literature where one type of pollen functions to the complete exclusion of the other. A gametophyte factor (Ga_1^s) in maize has been found (SCHWARTZ 1950) which is comparable in its action to S^{Ga}. When pollen carrying Ga_1^s and ga_1 compete on Ga_1^s styles, fertilization is accomplished solely by those gametes which carry the Ga_1^s allele. The self-sterility genes of Nicotiana, Oenothera, Trifolium, etc., may be placed in this category.

SUMMARY

The inheritance of a male sterile condition in *Zea mays* is shown to be conditioned by an interaction of three factors: (1) a dominant gene for male sterility (Ms_{21}), (2) a dominant suppressor of sterility (S^{Ga}) associated with a gametophyte effect, and (3) a specific " sterile " cytoplasm (□) essential for the expression of male sterility. Male sterile plants are of the constitution □ $Msms\ s^{ga}s^{ga}$. Due to complete selective fertilization, the suppressor gene segregates only when the heterozygote, $S^{Ga}s^{ga}$, is used as the pistillate parent.

The breeding behavior of two partially male sterile plants is analyzed and found to be consistent with the proposed hypothesis.

Evidence is presented that cytoplasmic inheritance is involved. Alternative hypotheses are discussed.

A new male sterile line, arising from a spontaneous mutation to the " sterile " cytoplasm, is described. Its breeding behavior is identical with that of the original male sterile.

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INHERITANCE OF POLYDACTYLY IN THE MOUSE[1]

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POLYDACTYLY of the hind foot, involving essentially a duplication of the first toe, has been reported by MURRAY (1932), FORTUYN (1939), HOLT (1945, 1948), HOLT and WRIGHT (1948), and CHASE (1946). These cases belong to the same general phenotype. Cases where polydactyly in mice occurs on the forefeet or where polydactyly is associated with another character have been briefly reviewed by HOLT (1945) and will not be considered here. Polydactyly in other animals is known, notably in man, cat, guinea pig, and fowl but will be discussed only briefly in this paper.

The polydactyly described by HOLT, FORTUYN, CHASE, and presumably MURRAY varies in expression as follows: slight thickening of the hallux, considerable thickening, long hallux, two or more nails, small extra toe between first and normal second, complete extra toe, two or even three complete or partial extra toes (figs. 1 and 2). In some cases the first and extra toe are at right angles or a more acute angle to the thickened tarsus. The right foot has a higher percentage of polydactyly but it may occur on the left and also bilaterally. There is some normal overlap. CHANG (1939) studied the embryology of the polydactyly in FORTUYN's strain. On the 12th or 13th day of embryonic development an increase in mesenchyme is seen as well as of epidermis on the medial side of the limb bud.

No genetic tests have been made between my polydactyl line and those of MURRAY, FORTUYN, or HOLT. The identity of the phenotypic variations, however, is obvious, even to the rare occurrence of polydactyl forefeet in HOLT's line and in mine. Certain crosses and analyses have been made with my line and will be presented in this paper. Data have been accumulated over a period of six and one half years and involve over 4,000 mice.

STRAINS

The polydactyl line (Poly) in my colony started with ♂ 22927, born July 1, 1944. There was an extra toe on the right hind foot. This mouse was of the second generation from survivors of a Survival Experiment. This experiment had been continued for two years and four months. It consisted of individual virgin females and males of the same age of inbred strains C57 B, BE, and L (to be described below) which were put together (20 in all) into a large cage. These animals and their offspring were subjected to cycles of feeding and starving. A population census was taken every two months. The

[1] The mouse colony on which the work was based has been maintained by the UNIVERSITY OF ILLINOIS and, since 1948, by BROWN UNIVERSITY and grants-in-aid from the AMERICAN CANCER SOCIETY, recommended by the COMMITTEE ON GROWTH of the NATIONAL RESEARCH COUNCIL.

population, of course, varied considerably (range from 90 to 7). At the end of the experiment the survivors at that time were removed, some being tested for genotypes to compare with the genes and the frequency which went into the experiment (unpublished). One set of survivors, a male and two females, was mated to start a new inbred line. The polydactyl occurred in the second generation of this type of mating, the result of a brother × sister mating. This polydactyl male was mated with a female of the L strain. All F_1 offspring were normal (14 ♀, 13 ♂). An F_1 female was then backcrossed to the original male. One polydactyl female and one polydactyl male were obtained out

FIGURE 1.—Litter of six 10-day-old mice of Poly line. Animals resting on a glass plate and photographed from below. Seven polydactyl hind feet are seen exhibiting some of the variations of expression.

of 16 offspring. From these the Poly line was developed by successive brother-sister matings and some selection. The early Survival line from which the original male was derived was continued but without selection for polydactyly. Another polydactyl male (a sib) was born later and two polydactyls which were half sibs were obtained in this line. Only occasional polydactyls occurred in later generations.

Poly strain individuals which were used in the principal crosses to be reported below were all of the sixth or later generation of successive brother-sister matings. The Wh strain was only in its fifth generation and the R_{III} strain in its third generation in this laboratory at the time they were used. Both strains were supposedly inbred previously. All other strains (C57 B,

BE, L, Zr) used were of the tenth or later generation of inbreeding. The Poly strain is of genotype *CCA/aBBDDPPSS*, segregating for agouti and non-agouti. The Wh strain is a dominant white line and was obtained from the Laboratory of DR. D. R. CHARLES, UNIVERSITY OF ROCHESTER. The C57 B strain (*CCaaBBDDPPSS*) is the highly inbred C57 Black strain, originally obtained from the JACKSON MEMORIAL LABORATORY, Bar Harbor, Maine. The BE strain (*CCAAbbddPPSS ey-1 ey-1 ey-2 ey-2*) is an anophthalmic subline of the Anoph B strain which was derived and inbred from the anophthalmics of DR. C. C. LITTLE of the JACKSON MEMORIAL LABORATORY. The

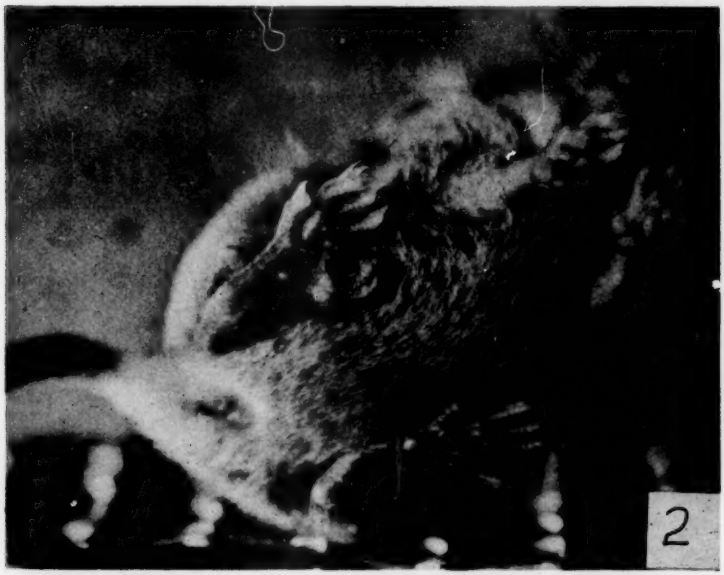

FIGURE 2.—Adult of Poly line. Animal resting on a glass plate and photographed from below. A typical expression of polydactyly on the right hind foot. The left hind foot is normal.

designation L has been given to an albino line (*ccaaBBDDPPSS*) inbred in my colony from a presumed Swiss ancestry. Zr is a line (*CCaabbDDppss*) inbred and selected for some recessive color genes and used largely as a testing stock. R_{III} is a standard albino strain and was obtained from the CURIE INSTITUTE in Paris. Two other inbred lines will be mentioned below in connection with the sporadic occurrence of polydactyly but they were not used in the main crosses. These were the silver strain, named Si, which was obtained from the colony of DR. W. H. GATES, Louisiana, and the yellow strain, named Y_p, which was obtained from the Jackson Laboratory. This latter strain was segregating for the pink-eye gene. A yellow/non-yellow line homozygous for *p* was derived.

RESULTS

Selection. As was mentioned above, the Poly line was derived from the original male of the second generation Survival line crossed with a female from strain L. A backcross was made to the original male and afterward only brother-sister matings were followed. Table 1 presents the history of this line.

TABLE 1

Selection of polydactyly.

Original mating is ♀ from strain L and ♂ from Survival line. Under "Mating Generation," 2-3,4 for instance means 3rd and 4th matings in second brother-sister generation. Term "sel" refers to matings which were made only if the whole litter from which the mating was derived was polydactylous; term "not sel" refers to cases after this practice had started in which not all of the litter was polydactylous. N = normal; P = polydactyl; BC = backcross. * One animal in which effect is on right front foot instead of hind foot.

Mating generation	Parents ♀ × ♂	From mating	Progeny ♀N	♀P	♂N	♂P	No.	%P
F_1	N × P	Orig.	14	....	13	....	27	0
BC to ♂ P	N × P	F_1 ♀ and orig. ♂	5	1	9	1	16	12.5
1-1	P × P	BC	17	6	22	8*	53	26.4
2-1	P × P	1-1	8	1	1	3	13	30.8
2-2	N × P	1-1	13	3	12	4	32	21.9
2-3,4	N × P*	1-1	4	3	4	9	20	60.0
3-1	N × P	2-1	9	2	8	9	28	39.3
3-4	N × P	2-2	8	6	10	8	32	43.8
4-1,2,3	N × P	3-1	22	12	13	18	65	46.2
4-4	N × P	3-4	10	3	14	16	43	44.2
4-5	P × P	3-4	6	4	8	4	22	36.4
5-1	P × P	4-4	....	2	....	3	5	100.0
5-2,3	N × P	4-4	9	17	11	13	50	60.0
5-4	N × P	4-2	3	2	6	....	11	18.2
5-5,6	P × P	4-2	6	3	6	6	21	42.9
6-1	P × P	5-5	1	16	1	8	26	92.3
7-1,2,3	P × P	6-1	4	16	5	13	38	76.3
sel 7-4,5,6	P × P	6-1	3	12	3	14	32	81.3
sel 8-1	P × P	7-5	5	30	2	23	60	88.3
8-3	N × P	7-5	3	2	....	2	7	57.1
sel 9-5,6,7,8	P × P	8-1	2	28	12	35	77	81.8
sel 10-1,4	P × P	9-5	2	8	....	9	19	89.5
sel 11-1,3	P × P	10-1	4	21	5	19	49	81.6
sel 12-1,2	P × P	11-3	5	20	4	17	46	80.4
sel 13-1,2,3,4	P × P	12-2	5	33	6	36	80	86.3
sel 14-7,8	P × P	13-3	1	10	3	15	29	86.2
sel 15-1,2	P × P	14-7	5	15	3	18	41	80.5
not sel 16-3,4	P × P	15-2	....	12	4	16	32	87.5
sel 16-5	P × P	15-1	2	14	2	12	30	86.7
not sel 17-1,2	P × P	16-3	5	10	2	16	33	78.8
not sel 17-3,4	P × P	16-4	2	9	1	10	22	86.4
sel 17-6,7	P × P	16-5	....	12	....	18	30	100.0
not sel 18-1,2	P × P	17-1	2	12	1	14	29	89.7
not sel 19-1,2	P × P	18-1	2	13	4	7	26	76.9
Totals			187	358	195	404	1144	

From generation 6 and on, excluding mating 8–3, the percentage of polydactyly from Poly × Poly matings is 84.5 percent (50 ♀ N, 291 ♀ P, 58 ♂ N, 300 ♂ P, total 699). The frequencies for each generation change, those before generation 6 being notably lower than those for generation 6 and later (table 1). No difference is found for males and females in the frequency or expression of this character. For the whole selection program there is no significant sex ratio difference (545 ♀, 599 ♂). Of the 762 polydactyls in this series, 8.66 percent are affected on both hind feet, 4.86 percent, on the left hind foot only, 86.35 percent, on the right hind foot only, and 0.13 percent (one mouse), on the right front foot only.

Little, if any, evidence of an age effect of the mother is apparent. Of 931 offspring born from generation 4 and on, the percentages with standard errors of polydactyls are as follows: 75.6 ± 2.4 for litters born when dams (and sires) one to 4 months of age; 77.3 ± 1.9, for 5 to 7 months of age; and 75.0 ± 3.6, for 8 to 11 months of age. Matings in this line usually were not continued to the reproductive limit since the practice has been to discontinue matings when the second generation beyond has been established. In the crosses to be mentioned below, however, matings generally were kept for the full reproductive span. In the reciprocal Poly × C57 B crosses, litters born when the parents were one year old or more have been compared with the litters of the same matings which were born earlier. The polydactyly in the earlier litters is 31.2 ± 2.2 percent (n = 426); in the later litters it is 25.6 ± 7.0 percent (n = 39). With the small numbers in the later litters, the difference of 5.6 percent is not statistically significant but is perhaps suggestive in view of HOLT's results to be discussed later. The last litters of the reproductive span usually are small, none over 5 here, and the effect, if any, may be associated with conditions which result in small litters.

Crosses. After the Poly line was well established, beginning especially with the sixth generation, certain crosses with other strains were made. These crosses are presented in table 2 and represent a total of 2,705 descendants.

Polydactyls occur in the F_1 generation of crosses with C57 B, BE, and L. They do not occur in the F_1's from crosses with R_{III}, Zr, and Wh but the numbers in these cases are small. In the combined reciprocal crosses with C57 B the frequency of polydactyly in the F_1's is 12 percent (n = 169) which is greater than for the other crosses. In the F_2 there is a frequency of 16 percent (n = 441) for the C57 B crosses which also is higher than for the other crosses, the closest being 14 percent (n = 118) for the cross with Wh. For the BE crosses the F_2 frequency is 8 percent (n = 290), for the R_{III} cross it is 7 percent (n = 100). For the L crosses the F_2 frequency is lower, 2 percent (n = 89). It should be recalled (table 1) that in the original establishment of the Poly line an outcross to the L line was involved.

In some crosses two types of F_2 matings were made, one using normal × normal F_1's, the other using normal × polydactyl F_1's. In the L cross no difference is discernible. In the C57 B and BE crosses, there is a difference. When combined, the percentage of polydactyl in F_2 from normal × normal matings is 8.0 percent (n = 460), in F_2 from normal × polydactyl matings, it

TABLE 2

Crosses with polydactyl line.

N = normal. P = polydactylous. BC = backcross generation from F_1 normal "segregant" mated back to "mutant" parent, exceptions are indicated. 2BC = second backcross. 3BC = third backcross. Original crosses are numbered, odd numbers for cases in which ♀ is of normal strain, even numbers for the reciprocal crosses.

Original cross	Generation	From parents ♀ × ♂	Progeny ♀N	♀P	♂N	♂P
1. C57 B ♀ × Poly ♂ (851 descendants)	F_1	N × P	51	5	48	8
	F_2	N × N	47	6	50	6
	F_2	N × P	43	12	29	8
	BC to ♂ P (sire)	N × P	57	48	60	45
	2BC to ♂ P (same ♂ as above)	N × P	6	5	3	14
	BC to ♀ P (♀ of Poly line)	P × N	24	10	21	13
	2BC to ♀ P (same ♀ as above)	P × N	4	11	8	11
	BC to ♀ N (orig. C57 B ♀)	N × N	27	1	32	....
	F_3 (from F_2 N × P)	N × P(+P × N)	13	29	18	45
	"	N × N	4	15	....	14
2. Poly ♀ × C57 B ♂ (297 descendants)	F_1	P × N	24	3	25	5
	F_2	N × N	48	5	47	6
	F_2	N × P	52	15	54	13
3. BE ♀ × Poly ♂ (415 descendants)	F_1	N × P	20	....	23	2
	F_2	N × N	68	4	75	6
	BC to ♂ P (sire)	N × P	23	8	17	9
	2BC to ♂ P (same ♂ as above)	N × P	35	20	47	25
	3BC to ♂ P (same ♂ as above)	N × P	3	6	3	10
	"2BC" to ♂ P (BC of P ♀ to same ♂ as above)	P × P	2	3	4	2
4. Poly ♀ × BE ♂ (157 descendants)	F_1	P × N	5	1	5	....
	F_2	N × N	43	2	45	2
	F_2	P × N	18	4	18	5
	BC to ♂ N (sire)	N × N	4	....	5	....
5. L ♀ × Poly ♂ (88 descendants)	F_1	N × P	16	1	15	....
	F_2	N × N	19	....	15	1
	F_2	P × N	15	.....	6	....
6. Poly ♀ × L ♂ (77 descendants)	F_1	P × N	4	....	5	....
	F_2	N × N	17	1	15	....
	BC to ♀ P (dam)	P × N	13	4	16	2

TABLE 2 *(continued)*

Original cross	Generation	From parents ♀×♂	Progeny ♀N	♀P	♂N	♂P
7. R_{III} ♀ × Poly ♂ (6 descendants)	F_1	N × P	....	....	6	....
8. Poly ♀ × R_{III} ♂ (113 descendants)	F_1	P × N	5	....	8	....
	F_2	N × N	37	4	56	3
9. Zr ♀ × Poly ♂ (53 descendants)	F_1	N × P	6	....	2	....
	BC to ♂ P (sire)	N × P	22	3	9	5
	"2BC" to ♂ P (BC of P ♀ to same ♂ as above)	P × P	2	1	3	....
12. Poly ♀ × Wh ♂ (648 descendants)	F_1	P × N	2	....	5	....
	F_2	N × N	59	10	43	6
	BC to ♀ P (dam)	P × N	4	13	9	3
	BC to ♀ N (♀ of Wh line)	N × N	6	....	13	....
	F_3	P × P	2	4	....	3
	"	N × P(+P × N)	56	20	54	16
	"	N × N	45	2	45	2
	F_4 (from F_3 N × P)	P × P	13	5	14	5
	F_4 (from F_3 P × N)	P × N	17	9	17	9
	F_4 (from F_3 N × N)	N × N	8	....	5	....
	F_5 (from F_4 P × N)	P × N	17	8	27	5
	F_5 (from F_4 P × N)	N × N	9	2	8	....
	F_6 (from F_5 P × N)	P × N	....	1	4	2
	F_6 (from F_5 P × N)	N × N	16	1	16	2
	F_7 (from F_6 N × N)	N × P	2	....	4	....

is 21.0 percent (n = 271). The difference of 13.0 ± 2.8 percent is statistically significant (P = < .0001). In the backcrosses (backcross of F_1 normal × polydactyl parent or polydactyl of the Poly line) the percentages of polydactyls are higher but agree in general with the comparable F_2 crosses. These percentages, based on smaller numbers, are 42 percent (n = 278) for C57 B, 55 percent (n = only 29) for Wh, 30 percent (n = 57) for BE, 21 percent (n = only 39) for Zr, and 17 percent (n = only 35) for L. In F_3 and subsequent matings, it is apparent that polydactyly can be readily selected for or against. In BC matings to the normal line, it is also apparent that polydactyly can be eliminated or reduced to a rare condition.

A critical test in genetics consists of successive backcrosses of normal " segregants " to the " mutant " line. Although numbers are not large (table 2), it is clear in the second backcrosses after the original C57 B cross and in the third backcross after the original BE cross that there is a pronounced increase in the percentage of polydactyly. The second backcross labelled " 2BC " in the BE cross and the Zr cross are cases where a polydactyl " segregant " instead of a normal " segregant " is used. The numbers are too small to warrant any conclusions but are not inconsistent with the comparable normal second backcrosses.

In terms of symmetry of expression, there is a difference as a result of the different crosses. The Poly line, as mentioned earlier, has a frequency of 8.7 percent bilateral, 4.9 percent left only, and 86.4 percent right only (n = 762). From the reciprocal BE crosses with Poly, there is a comparable frequency, 8.6 percent bilateral, 6.9 percent left, and 84.5 percent right (n = 109). From the Wh cross there is a frequency of 3.9 percent bilateral, 14.8 percent left, and 81.3 percent right (n = 128). From the reciprocal C57 B crosses, however, there is considerable difference; namely, 25.1 percent bilateral, 13.9 percent left, and 60.9 percent right (n = 353). No attempt at selection for a particular expression was made in these crosses.

Earlier crosses. The above crosses involve the Poly line after it had reached the sixth generation of brother-sister matings. Before that time the original polydactyls appearing in the Survival line were crossed with other lines. The results are presented in table 3. On the basis of such a distribution of the 231

TABLE 3

Early crosses with polydactyl mice.

Explanations similar to those of table 2.

Original cross	Generation	From parents ♀ × ♂	Progeny ♀N	♀P	♂N	♂P
1. C57 B ♀ × Poly ♂	F_1	N × P	13	....	9	....
	F_2	N × N	5	....	6	1
2. Poly ♀ × C57 B ♂	F_1	P × N	8	....	10	3
	F_2	N × P	21	5	26	4
3. BE ♀ × Poly ♂	F_1	N × P	8	....	15	....
	F_2	N × N	8	....	6	4
	BC to ♂ P (sire)	N × P	8	4	7	4
4. Poly ♀ × BE ♂	F_1	P × N	3	....	4	....
5. L ♀ × Poly ♂	F_1	N × P	14	....	13	....
	F_2	N × N	5	....	3	....
7. Zr ♀ × Poly ♂	F_1	N × P	2	....	2	....
	BC to ♂ P (sire)	N × P	6	1	2	1

animals, the suggestion was made (CHASE 1946) that polydactyly was a sex-linked recessive with incomplete penetrance.

Obviously, from the later crosses reported above, this hypothesis is incorrect. Furthermore, although 231 animals are involved, the critical generations are inadequate. These were the F_1 of cross 2 and the F_2's of crosses 1 and 3. Later litters from some of these matings and later additional crosses indicated the doubtfulness of sex-linkage. These data are not included in this paper.

Sporadic polydactyly. Polydactyly of the same appearance as already described in this paper has been observed in my colony in certain inbred lines. The frequency in all cases is less than 0.5 percent. A total of 8 has been observed in the C57 B (Jackson Laboratory subline 6) line; 8 in the Si line (silver from GATES); 13 in the Yp line (pink-eyed yellows from Jackson Laboratory); 4 in the Zr line; and one in the R_{III} line. These have all occurred after polydactyly was first noticed in the Survival offspring mentioned above. Previous to that time no routine observations were made for this character. Subsequently, however, all mice in the colony have been checked at birth (25,000) and at weaning for polydactyly. No cases have been observed in strain L but that line was discontinued shortly after the matings in table 2 were made. Similarly none have been observed in BE but that also was discontinued as such at nearly the same time. The anophthalmic genes of BE were incorporated into another line involving Zr. This new anophthalmic line, Zrd, has given 17 sporadic polydactyls.

Other foot abnormalities have been observed as sporadics. In the Survival line, which earlier gave the original polydactyly used in this study, there occurred in the 10th and 11th generations two cases of extreme hind leg displacement, one case being polydactyl on the left. In the Zrd line, two gross abnormalities occurred. In one, the digits of the left hind foot were missing but two tabs extended at right angles to the tarsus; the right hind foot resembled human lobster claw. In the other case, no digits were present on either hind foot and the right front foot was small with only three toes (not a case of being eaten off by a parent). All four cases were viable but proved to be sterile.

Polydactyly has occurred occasionally in later generations from crosses separate from the Poly line. The derived Zrd line, mentioned above, is an example. The insulin resistant line (KL) in my laboratory never showed polydactyly but after it was crossed with C57 B, four polydactyls have occurred sporadically in the second and later generations. In none of these cases were polydactyl individuals involved in the original cross.

No extensive investigations have been made on these sporadic polydactyls of the isogenic lines, of the crosses, or of the lines being developed from these crosses. Two cases, however, may be instructive. A sporadic polydactyl male of the Si line was crossed with a normal female of the Poly line. No polydactyls were obtained in 19 F_1's, and 3 were obtained in 88 F_2's. Such a result might well have been expected from a cross with a normal Si male, judging from crosses presented in table 2. A sporadic polydactyl male of the C57 B line was crossed with a polydactyl female of the Poly line. No poly-

dactyls were obtained in 14 F_1's, also a companion mating with a normal C57 B sister gave no polydactyls in the immediate or later generations.

DISCUSSION

From the similarity of expressions and the variations in incidence possible from different crosses, it appears certain that HOLT's polydactyly case (1945, 1948; HOLT and WRIGHT 1946) is essentially the same as mine and also that the polydactyl cases of MURRAY (1932) and of FORTUYN (1939) belong in this category. Standard genetic tests to determine if the same gene or locus is involved might be made but would be meaningless unless each type is actually due to a primary gene. In HOLT's (1945) selected line, 60.8 percent of the polydactyls were bilaterally affected. In my line only 8.7 percent of the polydactyls were so affected. In crosses with C57 B, however, 25.1 percent of the polydactyls were affected on both hind feet. The difference is clearly one of modifiers. A decrease in polydactyly with increasing age of mother was found by HOLT (1948) to occur from one type of mating, *i.e.*, what she called homozygous polydactyls with about 50 percent manifestation. The effect was not found from backcross and "intercross" matings. No inbred lines were available for her study. As reported above, no age effect was observed in my selection line but matings in that line were routinely discarded before the reproductive limit was approached. In F_1's and later generations from outcrosses, however, matings were continued for a longer period and there is possibly a slight, but with small numbers not statistically significant, tendency for the litters born after the parents were one year old to have a smaller percentage of polydactyly.

The inheritance of this polydactyly, involving the hallux, is in all probability not due to a single gene difference. HOLT (1945) assumed a recessive, *py*, with incomplete penetrance and based her conclusion on the absence of any polydactyls in the F_1 generations from nine outcrosses to different lines. Her numbers were small (as are some of mine), the only large F_1 generation ($n = 51$) being that from a cross with the dark-headed self line. Her F_2 and BC generations agree very closely with the variations obtained in mine. In her highest case of polydactyl manifestation in an F_2, 50 normal to 13 polydactyl (Pallid × Polydactyly), it seems very probable that she would have obtained polydactyly in the F_1 with larger numbers. There were only four mice in this F_1, all normal. On the basis of her concept of a recessive *py* gene, she, of course, considers 100 percent manifestation to have occurred when a backcross has 50 percent polydactyls, or an F_2 has 25 percent. HOLT's selection experiment was based on a polydactyl male from an unselected mating crossed with a female heterozygous for fidget from DR. GRÜNEBERG's fidget stock. This female was normal but polydactyly was known from that stock. In the F_1, 16 normals were obtained and in the F_2, 71 normals to 28 polydactyls. She concluded that this female supplied modifiers but not the *py* gene. Later (HOLT and WRIGHT 1946), she obtained polydactyly in the F_1 from a cross with a polydactyl from GRÜNEBERG's (1943) fidget stock. Also from later generations of leaden × pallid and from leaden by another stock, all

non-polydactyl, she obtained occasional polydactyly (like my sporadics from crosses mentioned above). MURRAY's polydactyly came from a strain which gave rise to leaden. A sporadic polydactyl from the leaden source crossed with HOLT's polydactyly gave 3 polydactyls out of 11 in the F_1 of one mating and none out of 12 in the F_1 of another mating. The occurrence of these F_1 polydactyls is interpreted by her as evidence that the identical locus is involved in the MURRAY and HOLT cases as well as in the GRÜNEBERG case. The evidence is presumptive and would be critical only if a single recessive gene had been firmly established in all cases.

In view of the general similarity of my results with those of HOLT, the possibility of using her conclusion of a single-factor autosomal recessive with modifiers has been seriously considered. Yet with certain modifiers brought into the cross and with adequate numbers in the F_1, it is clear that polydactyly can occur in the first generation hybrids. The hypothesis of a sex-linked recessive with low penetrance (CHASE 1946) can be discarded on the basis of HOLT's results and of the results reported in this paper. The hypothesis was suggested on the basis of insufficient critical numbers and on what appears now to have been a fortuitous expression in relation to sex. A partial sex-linked gene has been considered but this hypothesis is not confirmed by the total results. The possibility of a sex-linked dominant with incomplete penetrance is also not tenable.

That a gene may be called dominant or recessive depending on the modifiers is well known. For example, the gene for white spotting, *s*, is known in several mammals and is generally considered to be recessive. With the introduction of certain modifiers, however, there is a shift of dominance in the guinea pig such that *s* may be called an incomplete recessive or *S*, an incomplete dominant (WRIGHT and CHASE 1936). A similar shift of dominance has been studied for white spotting in the mouse (DUNN and CHARLES 1937). Considering *py* as an incomplete recessive, depending on modifiers, is possible but the proportion of F_2's that are polydactyl is obviously too low. It would require the assumption that with one dose of the gene the threshold for expression is occasionally exceeded and that with two doses, the threshold is also exceeded only occasionally. A moderate degree of penetrance must also be assumed.

Another possibility is that we are dealing with a completely dominant gene having low penetrance. A dominant gene of this sort would place it in a category presumed for polydactyly in man, cat, and fowl. The similarity between F_1 and normal × normal F_2 could be explained on this basis. In the reciprocal C57 B × Poly crosses the penetrance of heterozygotes (F_1) would be about 12 percent; in the BE × Poly crosses the penetrance would be about 5 percent. In the N × N F_2's a corresponding frequency of polydactyls would be obtained if the homozygous "polydactyls" had a slight increase in penetrance to balance approximately the lack of penetrance in homozygous "normals." In crosses where no F_1 polydactyls are found, the assumption would be that the penetrance (due to modifiers, of course) is extremely low and/or the numbers of animals are inadequate.

The possibilities discussed above all imply a major pair of alleles and various arrays of modifiers. With enough assumptions as to dominance and penetrance and with limited data, one or other of the interpretations can be applied. The results can be explained, however, on a different basis, namely, a multiplicity of factors, none of which is a major gene. In support of such a view are three primary points. First, the statistically significant difference between normal × normal F_2's and normal × polydactyl F_2's in the crosses with C57 B and with BE. This result implies that in the polydactyls used for the original crosses there was not complete homozygosity for polydactyly. These polydactyls were from the 6th to the 12th generation of successive brother-sister inbreeding. If this lack of homozygosity involved a major dominant or semi-dominant gene with low penetrance, then the similarity between F_1 and $N \times N$ F_2 frequencies would be difficult to explain though perhaps not impossible.

The second point which favors the view that there is no major pair of alleles involved is the occurrence of sporadic polydactyls in various inbred lines and in some crosses between these lines. It would seem that the threshold for polydactyly is exceeded occasionally (always less than 0.5 percent) in the inbred lines due to non-hereditary variability. This is comparable to the 8 percent eye reduction, mostly on the right side, in females of the C57 B line. The hereditary constitution of the strain is such that the threshold is exceeded in that frequency. Matings from animals with reduced eyes do not alter that frequency, *i.e.*, no parent/offspring correlation. The little evidence of this sort for sporadic polydactyly in isogenic lines appears to be the same. In crosses between inbred lines, new hereditary constitutions are obtained, some of which may be nearer the threshold to be exceeded by non-hereditary variation. HOLT's (1946) sporadics from leaden ancestry would be interpreted by me in this way rather than as having a specific gene, *py*.

The third point suggesting that no single major gene is involved is the evidence from successive backcrosses. The only sound test for single-factor inheritance is the maintenance of a 50–50 ratio in a series of successive backcrosses of normal "segregants" to the "mutant" line. In the results presented here (similar tests not made by HOLT), the percentage of polydactyly in the second and third backcrosses increases beyond that of the first backcross and, although the numbers are small, apparently exceeds greatly the expected 50 percent level (or 100 percent manifestation of HOLT). This last evidence clearly contradicts the possibility that polydactyly is a simple autosomal recessive. That polydactyly might be a dominant is still possible from such evidence but in backcrosses to the normal line the frequency is zero or less than 2 percent which is much less than might be expected on the basis of the associated F_1 and F_2 results.

The similarity of inheritance to one type of polydactyly in the guinea pig is obvious. In the guinea pig there are normally three toes on the hind foot, the polydactyly being the occurrence of a fourth post-axial toe, the little toe. This type, first described by CASTLE (1906), is quite different in kind from the mouse polydactyly, but the mode of inheritance has certain important

similarities. CASTLE described it as a dominant but not a simple dominant. Later, WRIGHT (1934) reported the result of crosses between the 4-toed strain that had been developed and various normal 3-toed strains. In one cross (polydactyl strain D × strain 2) there were no polydactyl F_1's and a 3 : 1 F_2 as well as a 1 : 1 backcross. In successive backcrosses, however, the frequency of polydactyly increased and the hypothesis of a single factor mendelian inheritance was proved incorrect. An estimate of three or more factors with no dominance was made in relation to a physiological threshold for an extra toe. Crosses of the polydactyl D strain with other normal strains gave some polydactyls in the F_1's and indicated that these normal strains were nearer the threshold than was strain 2.

An analysis of the results reported in this paper and of those of HOLT, indicate that the interpretation involving an autosomal recessive, *py*, is not tenable. A sex-linked recessive is also excluded. A dominant with low penetrance is possible but unlikely. Most probable is a situation involving several factors (first suggested by MURRAY 1932) with little dominance, and considerable non-hereditary variability, in relation to a threshold whereby additional mesenchyme is laid down mesially on the hind limb buds.

Such types of inheritance may be more general than is frequently assumed in mammals. Some cases of dominants with moderate or low penetrance, in man for example, could well be of this type. Likewise, certain recessives are so named mainly because a certain threshold has not been transcended in the hybrid. Where only outcrosses and occasional consanguinous matings are employed, as is normally necessary in man; and where only F_1's, F_2's, and occasional first backcrosses are employed, as in most other mammalian studies, the decision in terms of a major dominant or recessive is clearly open to question. The point of real importance is the existence of a physiological threshold, transcended or not by the hereditary and non-hereditary conditions prevailing. As geneticists, many of us, especially in mammalian work, have placed too much emphasis on the occurrence of mendelian 3 : 1 and 1 : 1 ratios and too little emphasis on the physiological conditions underlying such distributions. The terms "dominant" and "recessive" are meaningless except with relation to thresholds and all other genes and conditions prevailing. The description of this mouse polydactyly by MURRAY, FORTUYN and HOLT, and the study by CHANG of the embryology underlying it are more important than is the attempted identification of a "gene for it." In general, more important than the identification of "genes" is the identification of the character, the heritability of the character under prescribed conditions, and the threshold and physiological conditions underlying the character.

SUMMARY

1. Polydactyly involving the hallux of one or both hind feet in mice is described. The general similarity to the polydactyls of MURRAY, FORTUYN and HOLT is discussed. The degree of expression, including the amount of "normal overlap," varies. The right foot is more frequently affected than is the left.

2. Over 4000 mice are involved in this study. A selected line of polydactyls is described and the results of several crosses to normal inbred lines are presented. In certain crosses polydactyly occurs with low frequency in the F_1's, in other crosses it has not appeared.

3. Various possibilities as to the mode of inheritance are discussed. An autosomal recessive, *py*, with modifiers is found to be untenable as is also the possibility of a sex-linked recessive. A dominant with low penetrance is more difficult to disprove but is definitely questionable. In view of the evidence from sporadic polydactyls in several inbred lines, and especially the evidence from successive test backcrosses, the suggestion is made that polydactyly is the result of several hereditary factors, none of which is major, and considerable non-hereditary variability. All of this is in relation to a physiological threshold to be transcended for expression of polydactyly.

4. Implications for other studies of mammalian genetics are discussed.

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Page 514, table 5, (under Lac-V_1^r) for " 203 " read " 303 "
Page 518, table 6, (on line crossover region) for " ced " read " cef."

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Page 33, line 9, for " sternua " read " strenua."
Page 202, table 2, column 3, " 8," " 19," " 6," and " 6 " should each be preceded by " > ".
Page 305, line 4, for " **2:** 295–305 " read " **4:** 14–33."
Page 310, line 1, for " dba-2 " read " dba/2."
Page 560, line 33, for "(all sensitive to lambda)" read "(all sensitive to lambda-2)."

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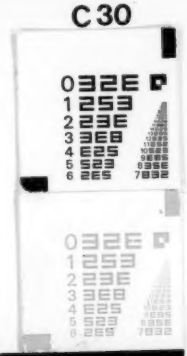
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